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				custom IPC display formats
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				IPC display formats
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MIDITO	0.1	3 DD	20	predefined hit display formats
NEWS		APR		EMBASE Controlled Term thesaurus enhanced
NEWS NEWS		APR MAY		IMSRESEARCH reloaded with enhancements INPAFAMDB now available on STN for patent family
CMTM	23	MAI	30	searching
NEWS	24	MZV	3.0	DGENE, PCTGEN, and USGENE enhanced with new homology
MEMP	4	1.1727.1	50	sequence search option
				sequence search operon
NEWS	EXPI	RESS	FEBI	RUARY 08 CURRENT WINDOWS VERSION IS V8.3,
112110				CURRENT DISCOVER FILE IS DATED 20 FEBRUARY 2008
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=> e endo y/au
                   ENDO WATARU/AU
           11
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E2
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          2561 --> ENDO Y/AU
E4
            8 ENDO Y DR/AU
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                  ENDO Y M/AU
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                  ENDO YAMAGAMI OSAMU/AU
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E.8
            1 ENDO YASAHARU/AU
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=> s e3-e7

L1 2996 ("ENDO Y"/AU OR "ENDO Y DR"/AU OR "ENDO Y I"/AU OR "ENDO Y M"/AU OR "ENDO YAETA"/AU)

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=> e sawasaki t/au
           1 SAWASAKI SUMIKO/AU
                                 SAWASAKI SUSUMU/AU
E3
                    91 --> SAWASAKI T/AU
                    11 SAWASAKI TACHIO/AU
3 SAWASAKI TAKA/AU
E4
                3 SAWASAKI TAKA/AU
1 SAWASAKI TAKAJI/AU
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11 SAWASAKI TAKASHI/AU
11 SAWASAKI TAKESHI/AU
3 SAWASAKI TAKURO/AU
19 SAWASAKI TATSUO/AU
201 SAWASAKI TATSUYA/AU
1 SAWASAKI TATUYA/AU
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=> s e3

L2 91 "SAWASAKI T"/AU

=> s e11-e12

L3 202 ("SAWASAKI TATSUYA"/AU OR "SAWASAKI TATUYA"/AU)

=> s 11-13

L4 3093 (L1 OR L2 OR L3)

=> s 14 AND ((protein(2a)synthes?) OR translation)(3a)((in(a)vitro) OR cell(a)free OR cell-free)

L5 215 L4 AND ((PROTEIN(2A) SYNTHES?) OR TRANSLATION)(3A)((IN(A) VITRO) OR

=> dup rem 15

PROCESSING COMPLETED FOR L5

L6 121 DUP REM L5 (94 DUPLICATES REMOVED)

=> s 16 AND (mrna OR template OR wheat OR atp)

L7 103 L6 AND (MRNA OR TEMPLATE OR WHEAT OR ATP)

=> d ibib ed abs 17 1-103

L7 ANSWER 1 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2008251993 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 18371187

TITLE: A set of ligation-independent in vitro translation vectors for

eukaryotic protein production.

AUTHOR: Bardoczy Viola; Geczi Viktoria; Sawasaki Tatsuya; Endo Yaeta;

Meszaros Tamas

CORPORATE SOURCE: Budapest University of Technology and Economics, Department of Applied Biotechnology and Food Science, 1111 Budapest, Muegyetem rkp. 3., Hungary..

bardoczy@mail.bme.hu

SOURCE: BMC biotechnology, (2008) Vol. 8, pp. 32. Electronic

Publication: 2008-03-27.

Journal code: 101088663. E-ISSN: 1472-6750.

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200805

ENTRY DATE: Entered STN: 17 Apr 2008

Last Updated on STN: 7 May 2008

Entered Medline: 6 May 2008

ED Entered STN: 17 Apr 2008

Last Updated on STN: 7 May 2008

Entered Medline: 6 May 2008

BACKGROUND: The last decade has brought the renaissance of protein studies and AΒ accelerated the development of high-throughput methods in all aspects of proteomics. Presently, most protein synthesis systems exploit the capacity of living cells to translate proteins, but their application is limited by several factors. A more flexible alternative protein production method is the cell-free in vitro protein translation. Currently available in vitro translation systems are suitable for high-throughput robotic protein production, fulfilling the requirements of proteomics studies. Wheat germ extract based in vitro translation system is likely the most promising method, since numerous eukaryotic proteins can be cost-efficiently synthesized in their native folded form. Although currently available vectors for wheat embryo in vitro translation systems ensure high productivity, they do not meet the requirements of state-of-the-art proteomics. Target genes have to be inserted using restriction endonucleases and the plasmids do not encode cleavable affinity purification tags. RESULTS: We designed four ligation independent cloning (LIC) vectors for wheat germ extract based in vitro protein translation. In these constructs, the RNA transcription is driven by T7 or SP6 phage polymerase and two TEV protease cleavable affinity tags can be added to aid protein purification. To evaluate our improved vectors, a plant mitogen activated protein kinase was cloned in all four constructs. Purification of this eukaryotic protein kinase demonstrated that all constructs functioned as intended: insertion of PCR fragment by LIC worked efficiently, affinity purification of translated proteins by GST-Sepharose or MagneHis particles resulted in high purity kinase, and the affinity tags could

efficiently be removed under different reaction conditions. Furthermore, high in vitro kinase activity testified of proper folding of the purified protein. CONCLUSION: Four newly designed in vitro translation vectors have been constructed which allow fast and parallel cloning and protein purification, thus representing useful molecular tools for high-throughput production of eukaryotic proteins.

L7 ANSWER 2 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2008102820 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 18164779

TITLE: Production of yeast tRNA (m(7)G46) methyltransferase (Trm8-

Trm82 complex) in a wheat germ cell-free translation system.

AUTHOR: Matsumoto Keisuke; Tomikawa Chie; Toyooka Takashi; Ochi Anna;

Takano Yoshitaka; Takayanagi Naoyuki; Abe Masato; Endo Yaeta; Hori Hiroyuki

CORPORATE SOURCE: Department of Applied Chemistry, Faculty of Engineering, Ehime

University, Bunkyo 3, Matsuyama 790-8577, Japan.

SOURCE: Journal of biotechnology, (2008 Feb 29) Vol. 133, No. 4, pp.

453-60. Electronic Publication: 2007-11-24.

Journal code: 8411927. ISSN: 0168-1656.

PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200805

ENTRY DATE: Entered STN: 12 Feb 2008

Last Updated on STN: 20 May 2008 Entered Medline: 19 May 2008

ED Entered STN: 12 Feb 2008

Last Updated on STN: 20 May 2008 Entered Medline: 19 May 2008

AΒ Cell-free translation systems are a powerful tool for the production of many kinds of proteins. However the production of proteins made up of hetero subunits is a major problem. In this study, we selected yeast tRNA (m(7)G46)methyltransferase (Trm8-Trm82 heterodimer) as a model protein. The enzyme catalyzes a methyl-transfer from S-adenosyl-l-methionine to the N(7) atom of guanine at position 46 in tRNA. When Trm8 or Trm82 mRNA were used for cellfree translation, Trm8 and Trm82 proteins could be synthesized. Upon mixing the synthesized Trm8 and Trm82 proteins, no active Trm8-Trm82 heterodimer was produced. Active Trm8-Trm82 heterodimer was only synthesized under conditions, in which both Trm8 and Trm82 mRNAs were co-translated. These results strongly suggest that the association of the Trm8 and Trm82 subunits is translationally controlled in living cells. Kinetic parameters of purified Trm8-Trm82 heterodimer were measured and these showed that the protein has comparable activity to other tRNA methyltransferases. The production of the m(7)G base at position 46 in tRNA was confirmed by two-dimensional thin layer chromatography and aniline cleavage of the methylated tRNA.

L7 ANSWER 3 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2007735707 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 17981875

TITLE: A cell-free translation and proteoliposome reconstitution

system for functional analysis of plant solute transporters.

AUTHOR: Nozawa Akira; Nanamiya Hideaki; Miyata Takuji; Linka Nicole;

Endo Yaeta; Weber Andreas P M; Tozawa Yuzuru

CORPORATE SOURCE: Cell-Free Science and Technology Research Center, and The

Venture Business Laboratory, Ehime University, Matsuyama, Japan.

SOURCE: Plant & cell physiology, (2007 Dec) Vol. 48, No. 12, pp. 1815-

20. Electronic Publication: 2007-11-02.

Journal code: 9430925. ISSN: 0032-0781.

PUB. COUNTRY: Japan

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200804

ENTRY DATE: Entered STN: 13 Dec 2007

Last Updated on STN: 2 Apr 2008

Entered Medline: 1 Apr 2008

ED Entered STN: 13 Dec 2007

Last Updated on STN: 2 Apr 2008 Entered Medline: 1 Apr 2008

AB We describe here a novel proteoliposome reconstitution system for functional analysis of plant membrane transporters that is based on a modified wheat germ cell-free translation system. We established optimized conditions for the reconstitution system with Arabidopsis thaliana phosphoenolpyruvate/phosphate translocator 1 (AtPPT1) as a model transporter. A high activity of AtPPT1 was achieved by synthesis of the protein in the presence of both a detergent such as Brij35 and liposomes. We also determined the substrate specificities of three putative rice PPT homologs with this system. The cell-free proteoliposome reconstitution system provides a valuable tool for functional analysis of transporter proteins.

L7 ANSWER 4 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2007704683 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 18029735

TITLE: Hetero subunit interaction and RNA recognition of yeast tRNA (m7G46) methyltransferase synthesized in a wheat germ cell-free translation system.

AUTHOR: Muneyoshi Yuki; Matsumoto Keisuke; Tomikawa Chie; Toyooka

Takashi; Ochi Anna; Masaoka Takashi; Endo Yaeta; Hori Hiroyuki

CORPORATE SOURCE: Department of Materials and Biotechnology, Graduate School of

Science and Engineering Ehime University, Matsuyama, 790-8577 Japan.

SOURCE: Nucleic acids symposium series (2004), (2007) No. 51, pp. 359-

60.

Journal code: 101259965. E-ISSN: 1746-8272.

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200804

ENTRY DATE: Entered STN: 29 Nov 2007

Last Updated on STN: 29 Apr 2008 Entered Medline: 28 Apr 2008

ED Entered STN: 29 Nov 2007

Last Updated on STN: 29 Apr 2008

Entered Medline: 28 Apr 2008

AB Yeast tRNA (m(7)G46) methyltransferase contains two protein subunits (Trm8 and Trm82). The enzyme catalyzes a methyl-transfer from S-adenosyl-L-methionine to the N(7) atom of guanine at position 46 in tRNA. We deviced synthesis of active Trm8-Trm82 heterodimer in a wheat germ cell-free translation system. When Trm8 or Trm82 mRNA were used for a synthesis, Trm8 or Trm82 protein could be synthesized. Upon mixing the synthesized Trm8 and Trm82 proteins, no active Trm8-Trm82 heterodimer was produced. Active Trm8-Trm82 heterodimer was only synthesized under conditions, in which both Trm8 and Trm82 mRNAs were cotranslated. To address the RNA recognition mechanism of the Trm8-Trm82 complex, we investigated methyl acceptance activities of eight truncated yeast

tRNA(Phe) transcripts. In this meeting, we demonstrate that yeast Trm8-Trm82 has stricter recognition requirements for the tRNA molecule as compared to the bacterial enzyme, TrmB.

L7 ANSWER 5 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2007418268 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 17634598

TITLE: Methods for high-throughput materialization of genetic

information based on wheat germ cell-free expression system.

AUTHOR: Sawasaki Tatsuya; Morishita Ryo; Gouda Mudeppa D; Endo Yaeta

CORPORATE SOURCE: Cell-Free Science and Technology Research Center, Ehime

University, Matsuyama, Japan.

SOURCE: Methods in molecular biology (Clifton, N.J.), (2007) Vol. 375,

pp. 95-106. Ref: 10

Journal code: 9214969. ISSN: 1064-3745.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

General Review; (REVIEW)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200708

ENTRY DATE: Entered STN: 20 Jul 2007

Last Updated on STN: 10 Aug 2007

Entered Medline: 9 Aug 2007

ED Entered STN: 20 Jul 2007

Last Updated on STN: 10 Aug 2007

Entered Medline: 9 Aug 2007

AB Among the cell-free protein synthesis systems, the wheat germ-based translation system has significant advantages for the high-throughput production of eukaryotic multidomain proteins in folded state. Here, we describe protocols for this cell-free expression system.

L7 ANSWER 6 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2007292338 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 17367182

TITLE: Construction of intramolecular luciferase complementation probe

for detecting specific RNA.

AUTHOR: Endoh Tamaki; Mie Masayasu; Funabashi Hisakage; Sawasaki

Tatsuya; Endo Yaeta; Kobatake Eiry

CORPORATE SOURCE: Department of Biological Information, Graduate School of Bioscience and Biotechnology, 4259, Nagatsuta, Yokohama, 226-8501, Japan.

SOURCE: Bioconjugate chemistry, (2007 May-Jun) Vol. 18, No. 3, pp. 956-

62. Electronic Publication: 2007-03-17.

Journal code: 9010319. ISSN: 1043-1802.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200708

ENTRY DATE: Entered STN: 17 May 2007

Last Updated on STN: 30 Aug 2007

Entered Medline: 29 Aug 2007

ED Entered STN: 17 May 2007

Last Updated on STN: 30 Aug 2007

Entered Medline: 29 Aug 2007

AB Intermolecular enzyme complementation assay is a useful method for detecting protein-protein interactions. Specifically, bioluminescent signals produced

from reconstructed split luciferase fragments are powerful tools for in vivo analysis because the bioluminescent signals have been visualized both in cultured cells and living animals. However, they are limited for detection and evaluation of biological events relevant to intermolecular protein-protein interactions. In this study, we constructed an intramolecular luciferase complementation probe for detecting target biomolecules other than proteinprotein interactions. It consists of peptide-inserted firefly luciferase (PI-FLuc) containing a short peptide between internally divided firefly luciferase. The inserted short peptide triggers FLuc complementation or discomplementation and subsequent reactivation or inactivation of FLuc activity through its induced fit conformational changes. We chose RNA binding arginine rich motif (ARM) peptides, Rev and/or Tat, for model peptide insertion, and expressed constructed PI-FLuc probe variants using a wheat germ cell-free protein synthesis system. They showed FLuc activity changes, reactivation, or inactivation after binding to their specific RNA targets. Furthermore, to expand the versatility of the PI-FLuc RNA detection system, we designed split-RNA probes built to reform the ARM peptide binding site in the presence of arbitrarily selected target-RNA. As a result, the target RNA was homogeneously detected by FLuc luminescent signals mediated by a cooperative function of the PI-FLuc and split-RNA probe sets.

ANSWER 7 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2007242104 MEDLINE Full-text

PubMed ID: 17332011 DOCUMENT NUMBER:

Novel protein fold discovered in the PabI family of restriction TITLE:

enzymes.

Miyazono Ken-ichi; Watanabe Miki; Kosinski Jan; Ishikawa Ken; AUTHOR:

Kamo Masayuki; Sawasaki Tatsuya; Nagata Koji; Bujnicki Janusz M; Endo Yaeta;

Tanokura Masaru; Kobayashi Ichizo

CORPORATE SOURCE: Department of Applied Biological Chemistry, Graduate School of

Agricultural and Life Sciences, University of Tokyo, Tokyo, Japan.

SOURCE: Nucleic acids research, (2007) Vol. 35, No. 6, pp. 1908-18.

Electronic Publication: 2007-03-01.

Journal code: 0411011. E-ISSN: 1362-4962.

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

OTHER SOURCE: PDB-2DVY ENTRY MONTH: 200705

ENTRY DATE: Entered STN: 25 Apr 2007

> Last Updated on STN: 8 May 2007 Entered Medline: 7 May 2007

ED Entered STN: 25 Apr 2007

Last Updated on STN: 8 May 2007

Entered Medline: 7 May 2007

AΒ Although structures of many DNA-binding proteins have been solved, they fall into a limited number of folds. Here, we describe an approach that led to the finding of a novel DNA-binding fold. Based on the behavior of Type II restriction-modification gene complexes as mobile elements, our earlier work identified a restriction enzyme, R.PabI, and its cognate modification enzyme in Pyrococcus abyssi through comparison of closely related genomes. While the modification methyltransferase was easily recognized, R.PabI was predicted to have a novel 3D structure. We expressed cytotoxic R.PabI in a wheat-germbased cell-free translation system and determined its crystal structure. R.PabI turned out to adopt a novel protein fold. Homodimeric R.PabI has a curved anti-parallel beta-sheet that forms a 'half pipe'. Mutational and in silico DNA-binding analyses have assigned it as the double-strand DNA-binding

site. Unlike most restriction enzymes analyzed, R.PabI is able to cleave DNA in the absence of Mg(2+). These results demonstrate the value of genome comparison and the wheat-germ-based system in finding a novel DNA-binding motif in mobile DNases and, in general, a novel protein fold in horizontally transferred genes.

L7 ANSWER 8 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2007240109 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 17447497

TITLE: Wheat germ cell-free protein synthesis.

AUTHOR: Endo Yaeta; Sawasaki Tatsuya

CORPORATE SOURCE: Cell-Free Science and Technology Research Center, Ehime

University, Matsuyama, Japan.

SOURCE: Seikagaku. The Journal of Japanese Biochemical Society, (2007

Mar) Vol. 79, No. 3, pp. 229-38. Ref: 32

Journal code: 0413564. ISSN: 0037-1017.

PUB. COUNTRY: Japan

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

General Review; (REVIEW)

LANGUAGE: Japanese

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200706

ENTRY DATE: Entered STN: 24 Apr 2007

Last Updated on STN: 13 Jun 2007 Entered Medline: 12 Jun 2007

ED Entered STN: 24 Apr 2007

Last Updated on STN: 13 Jun 2007 Entered Medline: 12 Jun 2007

L7 ANSWER 9 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2007219087 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 17348022

TITLE: Detection of structural changes in a cofactor binding protein by using a wheat germ cell-free protein synthesis system coupled with unnatural amino acid probing.

AUTHOR: Abe Masato; Ohno Satoshi; Yokogawa Takashi; Nakanishi Takeshi; Arisaka Fumio; Hosoya Takamitsu; Hiramatsu Toshiyuki; Suzuki Masaaki; Ogasawara Tomio; Sawasaki Tatsuya; Nishikawa Kazuya; Kitamura Masaya; Hori Hiroyuki;

Endo Yaeta

CORPORATE SOURCE: Department of Applied Chemistry, Faculty of Engineering, Ehime University, Matsuyama 790-8577, Japan.

SOURCE: Proteins, (2007 May 15) Vol. 67, No. 3, pp. 643-52.

Journal code: 8700181. E-ISSN: 1097-0134.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200707

ENTRY DATE: Entered STN: 13 Apr 2007

Last Updated on STN: 10 Jul 2007

Entered Medline: 9 Jul 2007

ED Entered STN: 13 Apr 2007

Last Updated on STN: 10 Jul 2007

Entered Medline: 9 Jul 2007

AB A cell-free protein synthesis system is a powerful tool with which unnatural amino acids can be introduced into polypeptide chains. Here, the authors describe unnatural amino acid probing in a wheat germ cell-free translation system as a method for detecting the structural changes that occur in a

cofactor binding protein on a conversion of the protein from an apo-form to a holo-form. The authors selected the FMN-binding protein from Desulfovibrio vulgaris as a model protein. The apo-form of the protein was synthesized efficiently in the absence of FMN. The purified apo-form could be correctly converted to the holo-form. Thus, the system could synthesize the active apoform. Gel filtration chromatography, analytical ultracentrifugation, and circular dichroism-spectra studies suggested that the FMN-binding site of the apo-form is open as compared with the holo-form. To confirm this idea, the unnatural amino acid probing was performed by incorporating 3-azido-L-tyrosine at the Tyr35 residue in the FMN-binding site. The authors optimized three steps in their system. The introduced 3-azido-L-tyrosine residue was subjected to specific chemical modification by a fluorescein-triarylphosphine derivative. The initial velocity of the apo-form reaction was 20 fold faster than that of the holo-form, demonstrating that the Tyr35 residue in the apoform is open to solvent. 2007 Wiley-Liss, Inc.

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L7 ANSWER 10 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2007015216 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 17123829

TITLE: Sequence specificity and efficiency of protein N-terminal

methionine elimination in wheat-embryo cell-free system.

AUTHOR: Kanno Takuya; Kitano Michiko; Kato Rika; Omori Akira; Endo

Yaeta; Tozawa Yuzuru

CORPORATE SOURCE: Cell-Free Science and Technology Research Center, Ehime

University, Matsuyama 790-8577, Japan.

SOURCE: Protein expression and purification, (2007 Mar) Vol. 52, No. 1,

pp. 59-65. Electronic Publication: 2006-09-20.

Journal code: 9101496. ISSN: 1046-5928.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200705

ENTRY DATE: Entered STN: 10 Jan 2007

Last Updated on STN: 10 May 2007

Entered Medline: 9 May 2007

ED Entered STN: 10 Jan 2007

Last Updated on STN: 10 May 2007

Entered Medline: 9 May 2007

Recent improvements in wheat-embryo cell-free translation resulted in a highly AB productive system for protein preparation. To clarify N-terminal processing of the cell-free system in a preparative-scale (> mg protein product per ml), 20 mutant variants of maltose-binding protein (MalE), each having a different penultimate residue in the sequence Met-Xaa-Ile-Glu-, and 20 glutathione Stransferase (GST) variants, having Met-Xaa-Pro-Ile-sequence, were designed and synthesized. The MalE and GST proteins were purified by amylose-resin and glutathione columns, respectively, followed by analysis of their N-terminal sequences. These investigations revealed that sequence specificity and efficiency of the N-terminal Met (N-Met) elimination in the cell-free system are similar to those reported from investigations in cellular systems or in the wheat-embryo cell-free protein expression system in analytical scale (approximately 10 microg protein product per ml). Cleavage of the N-Met is basically determined by the penultimate amino acid in the polypeptide sequence. In the case of MalE, the cleavage was efficient when the penultimate residue was Ala, Cys, Gly, Pro, Ser or Thr. But, in the case of GST with Pro as the antepenultimate residue, the efficiency was significantly reduced when the penultimate residue was Gly or Thr. We also confirmed that substitution of the antepenultimate residue in MalE to Pro drastically reduced

the efficiency of N-Met cleavage when the penultimate residue was Ala, Gly, Pro, Ser or Thr, indicating inhibitory effects of antepenultimate residue Pro on N-Met elimination. These results clarified sequence-specific functions of the endogenous N-terminal processing machinery in the scaled-up wheat-embryo cell-free translation system.

L7 ANSWER 11 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2006472580 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 16828277

TITLE: Cell-free expression systems for eukaryotic protein production.

AUTHOR: Endo Yaeta; Sawasaki Tatsuya

CORPORATE SOURCE: Cell-Free Science and Technology Research Center, Ehime

University, Matsuyama, Japan.. yendo@eng.ehime-u.ac.jp

SOURCE: Current opinion in biotechnology, (2006 Aug) Vol. 17, No. 4,

pp. 373-80. Electronic Publication: 2006-07-07. Ref: 41

Journal code: 9100492. ISSN: 0958-1669.

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

(RESEARCH SUPPORT, NON-U.S. GOV'T)

General Review; (REVIEW)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200610

ENTRY DATE: Entered STN: 10 Aug 2006

Last Updated on STN: 6 Oct 2006 Entered Medline: 5 Oct 2006

ED Entered STN: 10 Aug 2006

Last Updated on STN: 6 Oct 2006 Entered Medline: 5 Oct 2006

AB Following the success of genome sequencing projects, attention has now turned to studies of the structure and function of proteins. Although cell-based expression systems for protein production have been widely used, they have certain limitations in terms of the quality and quantity of the proteins produced and for high-throughput production. Many of these limitations can be circumvented by the use of cell-free translation systems. Among such systems, the wheat germ based system is of special interest for its eukaryotic nature; it has the significant advantage of producing eukaryotic multidomain proteins in a folded state. Several advances in the use of cell-free expression systems have been made in the past few years and successful applications of these systems to produce proteins for functional and structural biology studies have been reported.

L7 ANSWER 12 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2006375984 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 16708362

TITLE: Tolerance for random recombination of domains in prokaryotic and eukaryotic translation systems: Limited interdomain misfolding in a eukaryotic translation system.

AUTHOR: Hirano Nobutaka; Sawasaki Tatsuya; Tozawa Yuzuru; Endo Yaeta;

Takai Kazuyuki

CORPORATE SOURCE: Venture Business Laboratory, Ehime University, Ehime, Japan.

SOURCE: Proteins, (2006 Aug 1) Vol. 64, No. 2, pp. 343-54.

Journal code: 8700181. E-ISSN: 1097-0134.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200608

ENTRY DATE: Entered STN: 23 Jun 2006

Last Updated on STN: 3 Aug 2006 Entered Medline: 2 Aug 2006

ED Entered STN: 23 Jun 2006

Last Updated on STN: 3 Aug 2006 Entered Medline: 2 Aug 2006

It has been proposed that eukaryotic translation systems have a greater AΒ capacity for cotranslational folding of domains than prokaryotic translation systems, which reduces interdomain misfolding in multidomain proteins and, therefore, leads to tolerance for random recombination of domains. However, there has been a controversy as to whether prokaryotic and eukaryotic translation systems differ in the capacity for cotranslational domain folding. Here, to examine whether these systems differ in the tolerance for the random domain recombination, we systematically combined six proteins, out of which four are soluble and two are insoluble when produced in an Escherichia coli and a wheat germ cell- free protein synthesis systems, to construct a fusion protein library. Forty out of 60 two-domain proteins and 114 out of 120 three-domain proteins were more soluble when produced in the wheat system than in the E. coli system. Statistical analyses of the solubilities and the activities indicated that, in the wheat system but not in the E. coli system, the two soluble domains comprised mainly of beta-sheets tend to avoid interdomain misfolding and to fold properly even at the neighbor of the misfolded domains. These results demonstrate that a eukaryotic system permits the concomitance of a wider variety of domains within a single polypeptide chain than a prokaryotic system, which is probably due to the difference in the capacity for cotranslational folding. This difference is likely to be related to the postulated difference in the tolerance for random recombination of domains. Copyright 2006 Wiley-Liss, Inc.

L7 ANSWER 13 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2006307255 MEDLINE <u>Full-text</u>

DOCUMENT NUMBER: PubMed ID: 16714002

TITLE: In vitro selection of zinc finger DNA-binding proteins through

ribosome display.

AUTHOR: Ihara Hiroshi; Mie Masayasu; Funabashi Hisakage; Takahashi

Fumio; Sawasaki Tatsuya; Endo Yaeta; Kobatake Eiry

CORPORATE SOURCE: Department of Biological Information, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta, Midoriku, Yokohama 226-8501, Japan.

SOURCE: Biochemical and biophysical research communications, (2006 Jul

7) Vol. 345, No. 3, pp. 1149-54. Electronic Publication: 2006-05-12.

Journal code: 0372516. ISSN: 0006-291X.

PUB. COUNTRY: United States DOCUMENT TYPE: (IN VITRO)

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200608

ENTRY DATE: Entered STN: 1 Jun 2006

Last Updated on STN: 2 Aug 2006 Entered Medline: 1 Aug 2006

ED Entered STN: 1 Jun 2006

Last Updated on STN: 2 Aug 2006 Entered Medline: 1 Aug 2006

AB DNA-binding proteins with sequence specificities have a variety of applications. To create novel functional DNA-binding proteins, in vivo selection methods have been developed. There are, however, crucial problems with such methods, e.g., limitation of library size and difficulty of

expression of toxic proteins for the host cells. In order to overcome these problems, we developed a novel way to select DNA-binding proteins using an in vitro ribosome display technique. The three zinc finger DNA-binding protein libraries, based on a Zif268 containing randomized sequence in each finger, were prepared and transcribed to mRNA in vitro. The ternary ribosomal complexes, formed by mRNA, ribosome, and translated DNA-binding protein during translation in a rabbit reticulocyte in vitro translation system, were selected with biotinylated target DNA fragments bound to streptavidin magnetic beads. The extracted mRNAs from the selected complexes were amplified using reverse transcription PCR and then sequenced. This is the first report of the selection of DNA-binding proteins involving an in vitro ribosome display technique.

L7 ANSWER 14 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2005663866 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 16350953

TITLE: Advances in genome-wide protein expression using the wheat germ

cell-free system.

AUTHOR: Endo Yaeta; Sawasaki Tatsuya

CORPORATE SOURCE: Cell-Free Science and Technology Research Center, Ehime

University, Matsuyama, Japan.

SOURCE: Methods in molecular biology (Clifton, N.J.), (2005) Vol. 310,

pp. 145-67.

Journal code: 9214969. ISSN: 1064-3745.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200601

ENTRY DATE: Entered STN: 18 Dec 2005

Last Updated on STN: 24 Jan 2006 Entered Medline: 23 Jan 2006

ED Entered STN: 18 Dec 2005

Last Updated on STN: 24 Jan 2006 Entered Medline: 23 Jan 2006

In the current post-genomic era, cell-free translation platforms are gaining AΒ importance in structural as well as functional genomics. They are based on extracts prepared from Escherichia coli cells, wheat germ, or rabbit reticulocytes, and when programmed with any mRNA in the presence of energy sources and amino acids, can synthesize the respective protein in vitro. Among the cell-free systems, the wheat germ-based translation system is of special interest due to its eukaryotic nature and robustness. This chapter outlines the existing protein production platforms and their limitations, and describes the basic concept of the wheat germ-based cell-free system. It also demonstrates how the conventional wheat germ system can be improved by eliminating endogenous inhibitors, by using an expression vector specially designed for this system and polymerase chain reaction-directed protein synthesis directly from cDNAs in a bi-layer translation system. Finally, a robotic procedure for translation based on the wheat germ extract and bi-layer cell-free translation is described.

L7 ANSWER 15 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2005634530 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 16213724

TITLE: Selection of 5'-untranslated sequences that enhance initiation

of translation in a cell-free protein synthesis system from wheat embryos.

AUTHOR: Kamura Nami; Sawasaki Tatsuya; Kasahara Yuko; Takai Kazuyuki;

Endo Yaeta

CORPORATE SOURCE: Department of Applied Chemistry, Faculty of Engineering, Ehime

University, 3, Bunkyo-cho, Matsuyama, Ehime 790-8577, Japan.

SOURCE: Bioorganic & medicinal chemistry letters, (2005 Dec 15) Vol.

15, No. 24, pp. 5402-6. Electronic Publication: 2005-10-05.

Journal code: 9107377. ISSN: 0960-894X.

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200606

ENTRY DATE: Entered STN: 1 Dec 2005

Last Updated on STN: 28 Jun 2006 Entered Medline: 27 Jun 2006

ED Entered STN: 1 Dec 2005

Last Updated on STN: 28 Jun 2006 Entered Medline: 27 Jun 2006

AB Random libraries of mRNA 5'-leader sequences were screened to obtain some sequences that can stimulate the translation initiation in a cell-free translation system from wheat embryos as efficiently as the Omega sequence from tobacco mosaic virus. Several sequences that are as useful as the Omega sequence and are homologous to no known sequences survived the screening. We expect that these sequences add useful options to the cell-free protein synthesis system that is becoming a powerful tool in the post-genomic researches.

L7 ANSWER 16 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2005536985 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 16157309

TITLE: Activity-based in vitro selection of T4 DNA ligase.

AUTHOR: Takahashi Fumio; Funabashi Hisakage; Mie Masayasu; Endo Yaeta;

Sawasaki Tatsuya; Aizawa Masuo; Kobatake Eiry

CORPORATE SOURCE: Department of Biological Information, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta,

Midoriku, Yokohama 226-8501, Japan.

SOURCE: Biochemical and biophysical research communications, (2005 Oct

28) Vol. 336, No. 3, pp. 987-93.

Journal code: 0372516. ISSN: 0006-291X.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200511

ENTRY DATE: Entered STN: 12 Oct 2005

Last Updated on STN: 3 Nov 2005 Entered Medline: 1 Nov 2005

ED Entered STN: 12 Oct 2005

Last Updated on STN: 3 Nov 2005

Entered Medline: 1 Nov 2005

AB Recent in vitro methodologies for selection and directed evolution of proteins have concentrated not only on proteins with affinity such as single-chain antibody but also on enzymes. We developed a display technology for selection of T4 DNA ligase on ribosome because an in vitro selection method for DNA ligase had never been developed. The 3' end of mRNA encoding the gene of active or inactive T4 DNA ligase-spacer peptide fusion protein was hybridized to dsDNA fragments with cohesive ends, the substrate of T4 DNA ligase. After in vitro translation of the mRNA-dsDNA complex in a rabbit reticulocyte system, a mRNA-dsDNA-ribosome-ligase complex was produced. T4 DNA ligase enzyme displayed on a ribosome, through addition of a spacer peptide, is able

to react with dsDNA in the complex. The complex expressing active ligase was biotinylated by ligation with another biotinylated dsDNA probe and selected with streptavidin-coated magnetic beads. We effectively selected active T4 DNA ligase from a small amount of protein. The gene of the active T4 DNA ligase was enriched 40 times from a mixture of active and inactive genes using this selection strategy. This ribosomal display strategy may have high potential to be useful for selection of other enzymes associated with DNA.

L7 ANSWER 17 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2004567638 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 15452433

TITLE: A novel way of amino acid-specific assignment in (1)H-(15)N

HSQC spectra with a wheat germ cell-free protein synthesis system.

AUTHOR: Morita Eugene Hayato; Shimizu Masato; Ogasawara Tomio; Endo

Yaeta; Tanaka Rikou; Kohno Toshiyuki

CORPORATE SOURCE: Division of Gene Research, Department of Molecular Science, Integrated Center for Science, Ehime University, 3-5-7 Tarumi, Ehime 790-8566,

Japan.. ehmorita@dpc.ehime-u.ac.jp

SOURCE: Journal of biomolecular NMR, (2004 Sep) Vol. 30, No. 1, pp. 37-

45.

Journal code: 9110829. ISSN: 0925-2738.

PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200503

ENTRY DATE: Entered STN: 16 Nov 2004

Last Updated on STN: 12 Mar 2005 Entered Medline: 11 Mar 2005

ED Entered STN: 16 Nov 2004

Last Updated on STN: 12 Mar 2005 Entered Medline: 11 Mar 2005

For high-throughput protein structural analyses, it is indispensable to AB develop a reliable protein overexpression system. Although many protein overexpression systems, such as ones utilizing E. coli cells, have been developed, a lot of proteins functioning in solution still were synthesized as insoluble forms. Recently, a novel wheat germ cell-free protein synthesis system was developed, and many of such proteins were synthesized as soluble forms. This means that the applicability of this protein synthesis method to determination of the functional structures of soluble proteins. In our previous work, we synthesized (15)N-labeled proteins with this wheat germ cell-free system, and confirmed this applicability on the basis of the strong similarity between the (1)H-(15)N HSQC spectra for native proteins and the corresponding ones for synthesized ones. In this study, we developed a convenient and reliable method for amino acid selective assignment in (1)H-(15)N HSQC spectra of proteins, using several inhibitors for transaminases and glutamine synthase in the process of protein synthesis. Amino acid selective assignment in (1)H-(15)N HSQC spectra is a powerful means to monitor the features of proteins, such as folding, intermolecular interactions and so on. This is also the first direct experimental evidence of the presence of active transaminases and glutamine synthase in wheat germ extracts.

L7 ANSWER 18 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2004466577 MEDLINE <u>Full-text</u>

DOCUMENT NUMBER: PubMed ID: 15376967

TITLE: In vitro protein synthesis system: cell-free protein synthesis

system prepared from wheat germ.

AUTHOR: Sawasaki Tatsuya; Endo Yaetasawasaki@eng.ehime-u.ac.jp
SOURCE: Tanpakushitsu kakusan koso. Protein, nucleic acid, enzyme,

(2004 Aug) Vol. 49, No. 11 Suppl, pp. 1514-9. Ref: 12

Journal code: 0413762. ISSN: 0039-9450.

PUB. COUNTRY: Japan

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

General Review; (REVIEW)

LANGUAGE: Japanese

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200411

ENTRY DATE: Entered STN: 21 Sep 2004

Last Updated on STN: 3 Nov 2004

Entered Medline: 2 Nov 2004

ED Entered STN: 21 Sep 2004

Last Updated on STN: 3 Nov 2004 Entered Medline: 2 Nov 2004

L7 ANSWER 19 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2004375184 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 15276451

TITLE: Genome-scale, biochemical annotation method based on the wheat

germ cell-free protein synthesis system.

AUTHOR: Sawasaki Tatsuya; Hasegawa Yoshinori; Morishita Ryo; Seki

Motoaki; Shinozaki Kazuo; Endo Yaeta

CORPORATE SOURCE: Cell-Free Science and Technology Research Center, The Venture

Business Laboratory, Ehime University, Matsuyama 790-8577, Japan.

SOURCE: Phytochemistry, (2004 Jun) Vol. 65, No. 11, pp. 1549-55.

Journal code: 0151434. ISSN: 0031-9422.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200412

ENTRY DATE: Entered STN: 28 Jul 2004

Last Updated on STN: 19 Dec 2004

Entered Medline: 7 Dec 2004

ED Entered STN: 28 Jul 2004

Last Updated on STN: 19 Dec 2004

Entered Medline: 7 Dec 2004

Since the complete genomic DNA sequencing of various species, attention has AΒ turned to the structural properties, and functional characteristics of proteins. Current cell-free protein expression systems from eukaryotes are capable of synthesizing proteins with high speed and accuracy; however, the yields are low due to their instability over time. This report reviews the high-throughput, genome-scale biochemical annotation method based on the cellfree system prepared from wheat embryos. We first briefly reviewed our highly efficient and robust wheat germ cell-free protein synthesis system, and then showed an application of the system for materialization and characterization of genetic information taking a cDNA library of protein kinase from Arabidopsis thaliana as an example. The procedure consists of: (1) fusion of the gene-of-interest to a purification-tag, amplified by the split-primer PCR method; (2) transcription and purification of mRNA; (3) cell-free protein synthesis in the bilayer system using 96-well titer plate; (4) affinity purification and activity measurement. We took 439 cDNAs encoding kinases among 1064 genes annotated so far, and they were translated in parallel into protein. Subsequent assay revealed 207 products having autophosphorylation activity. Furthermore, seven proteins out of 26 calcium-dependent protein kinase genes tested did phosphorylate a synthetic peptide substrate in the presence of calcium ion, demonstrating that the translation products, retained

their substrate specificity. The information on biochemical function of gene products accumulated should revolutionize our understanding of biology and fundamentally alter the practice of medicine and influence other industries as well.

L7 ANSWER 20 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2004151429 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 15044017

TITLE: Formation of circular polyribosomes in wheat germ cell-free

protein synthesis system.

AUTHOR: Madin Kairat; Sawasaki Tatsuya; Kamura Nami; Takai Kazuyuki; Ogasawara Tomio; Yazaki Kazumori; Takei Toshiaki; Miura Kin-Ichiro; Esdo Yaeta CORPORATE SOURCE: Cell-free Science and Technology Research Center, and The Venture Business Laboratory, Ehime University, Matsuyama 790-8577, Japan.

SOURCE: FEBS letters, (2004 Mar 26) Vol. 562, No. 1-3, pp. 155-9.

Journal code: 0155157. ISSN: 0014-5793.

PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200405

ENTRY DATE: Entered STN: 27 Mar 2004

Last Updated on STN: 12 May 2004 Entered Medline: 11 May 2004

ED Entered STN: 27 Mar 2004

Last Updated on STN: 12 May 2004 Entered Medline: 11 May 2004

AB We report a morphological study of functioning ribosomes in a efficient and robust cell-free protein synthesis system prepared from wheat embryos. Sucrose density gradient analysis of translated mixtures programmed with luciferase mRNAs having different 5' and 3' untranslated regions showed formation of large polysomes. Electron microscopic examination of translation mixtures programmed with those of capped and polyadenylated mRNA revealed that ribosomes assemble into a circular-type polysome in vitro. Furthermore, a series of experiments using mRNAs lacking either cap, poly(A) tail or both also resulted in the formation of circular polysomes, which are indistinguishable from those with the original mRNA. The wheat germ cell-free system may provide a good experimental system for understanding functional ribosomes at the molecular level.

L7 ANSWER 21 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2003594215 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 14675755

TITLE: RALyase; a terminator of elongation function of depurinated

ribosomes.

AUTHOR: Ozawa Akihiko; Sawasaki Tatsuya; Takai Kazuyuki; Uchiumi

Toshio; Hori Hiroyuki; Endo Yaeta

CORPORATE SOURCE: Department of Applied Chemistry, Faculty of Engineering, Ehime University, Matsuyama, 790-8577, Japan.

SOURCE: FEBS letters, (2003 Dec 18) Vol. 555, No. 3, pp. 455-8.

Journal code: 0155157. ISSN: 0014-5793.

PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200401

ENTRY DATE: Entered STN: 17 Dec 2003

Last Updated on STN: 24 Jan 2004

Entered Medline: 23 Jan 2004

ED Entered STN: 17 Dec 2003

Last Updated on STN: 24 Jan 2004 Entered Medline: 23 Jan 2004

AB Plant ribosomal RNA apurinic site specific lyase (RALyase) cleaves the phosphodiester bond at the depurinated site produced by ribosome-inactivating protein, while the biological role of this enzyme is not clear. As the depurinated ribosomes retain weak translation elongation activities, it was suggested that RALyase completes the ribosome inactivation. To confirm this point, we measured the effects of the phosphodiester cleavage using a fusion of wheat RALyase produced with a cell-free protein synthesis system from wheat germ. The results indicated that RALyase diminishes the residual elongation activities of the depurinated ribosomes.

L7 ANSWER 22 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2003543983 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 14622267

TITLE: Efficient synthesis of a disulfide-containing protein through a

batch cell-free system from wheat germ.

AUTHOR: Kawasaki Takayasu; Gouda Mudeppa D; Sawasaki Tatsuya; Takai

Kazuyuki; Endo Yaeta

CORPORATE SOURCE: Cell-free Science and Technology Research Center, Ehime

University, Matsuyama, Japan.

SOURCE: European journal of biochemistry / FEBS, (2003 Dec) Vol. 270,

No. 23, pp. 4780-6.

Journal code: 0107600. ISSN: 0014-2956.

PUB. COUNTRY: Germany, Federal Republic of
DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200401

ENTRY DATE: Entered STN: 19 Nov 2003

Last Updated on STN: 6 Jan 2004 Entered Medline: 5 Jan 2004

ED Entered STN: 19 Nov 2003

Last Updated on STN: 6 Jan 2004 Entered Medline: 5 Jan 2004

We have developed a highly productive cell-free protein synthesis system from AΒ wheat germ, which is expected to become an important tool for postgenomic research. However, this system has not been optimized for the synthesis of disulfide-containing proteins. Thus, we searched here for translation conditions under which a model protein, a single-chain antibody variable fragment (scFv), could be synthesized into its active form. Before the start of translation, the reducing agent dithiothreitol, which normally is added to the wheat germ extract but which inhibits disulfide formation during translation, was removed by gel filtration. When the scFv mRNA was incubated with this dithiothreitol-deficient extract, more than half of the synthesized polypeptide was recovered in the soluble fraction. By addition of protein disulfide isomerase in the translation solution, the solubility of the product was further improved, and nearly half of the soluble polypeptides strongly bound to the antigen immobilized on an agarose support. This strong binding component had a high affinity as shown by surface-plasmon resonance analysis. These results show that the wheat germ cell-free system can produce a functional scFv with a simple change of the reaction ingredients. We also discuss protein folding in this system and suggest that the disulfide bridges are formed cotranslationally. Finally, we show that biotinylated scFv could be synthesized in similar fashion and immobilized on a solid surface to which

streptavidin is bound. SPR measurements for detection of antigens were also possible with the use of this immobilized surface.

L7 ANSWER 23 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2003485228 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 14563476

TITLE: High-throughput, genome-scale protein production method based

on the wheat germ cell-free expression system.

AUTHOR: Endo Yaeta; Sawasaki Tatsuya

CORPORATE SOURCE: Department of Applied Chemistry, Faculty of Engineering, Ehime

University, Matsuyama790-8577, Japan.. yendo@en3.ehime-u.ac.jp

SOURCE: Biotechnology advances, (2003 Nov) Vol. 21, No. 8, pp. 695-713.

Ref: 39

Journal code: 8403708. ISSN: 0734-9750.

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

General Review; (REVIEW)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200407

ENTRY DATE: Entered STN: 18 Oct 2003

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Entered Medline: 6 Jul 2004

ED Entered STN: 18 Oct 2003

Last Updated on STN: 7 Jul 2004

Entered Medline: 6 Jul 2004

Cell-free protein synthesis systems can synthesize proteins with high speed AΒ and accuracy, but produce only a low yield because of their instability over time. Here we review our recent advances in a cell-free protein synthesis system prepared from wheat embryos. We first addressed and resolved the source of the instability of existing systems in light of endogenous ribosomeinactivating proteins. We found that conventional wheat germ extracts contained the RNA N-glycosidase tritin and other inhibitors such as thionin, ribonucleases, deoxyribonucleases, and proteases that originate from the endosperm and inhibit translation. Extensive washing of wheat embryos to eliminate endosperm contaminants has resulted in extracts with a high degree of stability and activity. To maximize the translation yield and throughput of the system, we then focused on developing the following issues: optimization of the ORF flanking regions, a new strategy to construct PCRgenerated DNAs for screening, and design of an expression vector for largescale protein production. The resulting system achieves high-throughput expression, with a PCR-directed system at least 50 genes that can be translated in parallel, yielding between 0.1 and 2.3 mg of protein by one person within 2 days. Under the dialysis mode of reaction, the system with the expression vector can maintain productive translation for 14 days. The cell-free system described here bypasses most of the biological processes and lends itself to robotic automation for high-throughput expression of genetic information, thus opening up many possibilities in the post-genome era.

L7 ANSWER 24 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2003369546 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 12903243

TITLE: Construction of an efficient expression vector for coupled

transcription/translation in a wheat germ cell-free system.

AUTHOR: Sawasaki T; Hasegawa Y; Tsuchimochi M; Kasahara Y; Endo Y
CORPORATE SOURCE: Department of Applied Chemistry, Faculty of Engineering, Ehime

University, Matsuyama 790-8577, Japan.

SOURCE: Nucleic acids symposium series, (2000) No. 44, pp. 9-10.

Journal code: 8007206. ISSN: 0261-3166.

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200310

ENTRY DATE: Entered STN: 8 Aug 2003

Last Updated on STN: 3 Oct 2003

Entered Medline: 2 Oct 2003

ED Entered STN: 8 Aug 2003

Last Updated on STN: 3 Oct 2003 Entered Medline: 2 Oct 2003

AB Using the expression vector, pEU, which we have constructed, highly efficient in vitro protein synthesis can be achieved: The system works for 150 hours and without further template addition once the reaction has started, yielding 5 mg of enzymatically active protein in a 1 ml reaction.

L7 ANSWER 25 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2003369492 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 12903189

TITLE: Identification of Aquifex aeolicus tRNA (m2(2G26)

methyltransferase gene.

AUTHOR: Takeda Hiroshi; Hori Hiroyuki; Endo Yaeta

CORPORATE SOURCE: Department of Applied Chemistry, Faculty of Engineering, Ehime

University, Matsuyama 790-8577, Japan.

SOURCE: Nucleic acids research. Supplement (2001), (2002) No. 2, pp.

229-30.

Journal code: 101169367.

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200309

ENTRY DATE: Entered STN: 8 Aug 2003

Last Updated on STN: 9 Sep 2003 Entered Medline: 8 Sep 2003

ED Entered STN: 8 Aug 2003

Last Updated on STN: 9 Sep 2003 Entered Medline: 8 Sep 2003

The modifications of N2,N2-dimethylguanine (m2(2)G) are found in tRNAs and rRNAs from eukarya and archaea. In tRNAs, modification at position G26 is generated by tRNA (m2(2)G26) methyltransferase, which is encoded by the corresponding gene, trm1. This enzyme catalyzes the methyl-transfer from S-adenosyl-L-methionine to the semi-conserved residue, G26, via the intermediate modified base, m2G26. Recent genome sequencing project has been reported that the putative trm1 is encoded in the genome of Aquifex aeolicus, a hyper-thermophilic eubacterium as only one exception among eubacteria. In order to confirm whether this bacterial trm1 gene product is a real tRNA (m2(2)G26) methyltransferase or not, we expressed this protein by wheat germ in vitro cell-free translation system. Our biochemical analysis clearly showed that this gene product possessed tRNA (m2(2)G26) methyltransferase activity.

L7 ANSWER 26 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2003356040 MEDLINE <u>Full-text</u>

DOCUMENT NUMBER: PubMed ID: 12888530

TITLE: Highly stable and efficient mRNA templates for mRNA-protein

fusions and C-terminally labeled proteins.

AUTHOR: Miyamoto-Sato Etsuko; Takashima Hideaki; Fuse Shinichiro; Sue

Kaori; Ishizaka Masamichi; Tateyama Seiji; Horisawa Kenichi; Sawasaki Tatsuya; Endo

Yaeta; Yanagawa Hiroshi

CORPORATE SOURCE: Department of Biosciences and Informatics, Faculty of Science and Technology, Keio University, 3-14-1, Hiyoshi, Kohoku-ku, Yokohama 223-8522, Japan.

SOURCE: Nucleic acids research, (2003 Aug 1) Vol. 31, No. 15, pp. e78.

Journal code: 0411011. E-ISSN: 1362-4962.

PUB. COUNTRY: England: United Kingdom

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200308

ENTRY DATE: Entered STN: 31 Jul 2003

Last Updated on STN: 21 Aug 2003 Entered Medline: 20 Aug 2003

ED Entered STN: 31 Jul 2003

Last Updated on STN: 21 Aug 2003 Entered Medline: 20 Aug 2003

For high-throughput in vitro protein selection using genotype (mRNA)-phenotype AΒ (protein) fusion formation and C-terminal protein labeling as a post-selection analysis, it is important to improve the stability and efficiency of mRNA templates for both technologies. Here we describe an efficient single-strand ligation (90% of the input mRNAs) using a fluorescein-conjugated polyethylene glycol puromycin (Fluor-PEG Puro) spacer. This ligation provides a stable cjun mRNA with a flexible Fluor-PEG Puro spacer for efficient fusion formation (70% of the input mRNA with the PEG spacer) in a cell-free wheat germ translation system. When using a 5' untranslated region including SP6 promoter and Omega29 enhancer (a part of tobacco mosaic virus Omega), an A(8) sequence (eight consecutive adenylate residues) at the 3' end is suitable for fusion formation, while an XA(8) sequence (XhoI and the A(8) sequence) is suitable for C-terminal protein labeling. Further, we report that  ${\tt Fluor-PEG}$ N-t-butyloxycarbonylpuromycin [Puro(Boc)] spacer enhances the stability and efficiency of c-jun mRNA template for C-terminal protein labeling. These mRNA templates should be useful for puromycin-based technologies (fusion formation and C-terminal protein labeling) to facilitate high-throughput in vitro protein selection for not only evolutionary protein engineering, but also proteome exploration.

L7 ANSWER 27 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2003258238 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 12761392

TITLE: A wheat germ cell-free system is a novel way to screen protein

folding and function.

AUTHOR: Morita Eugene Hayato; Sawasaki Tatsuya; Tanaka Rikou; Endo

Yaeta; Kohno Toshiyuki

CORPORATE SOURCE: Center for Gene Research, Ehime University, Ehime 790-8566,

Japan.. ehmorita@dpc.ehime-u.ac.jp

SOURCE: Protein science: a publication of the Protein Society, (2003

Jun) Vol. 12, No. 6, pp. 1216-21.

Journal code: 9211750. ISSN: 0961-8368.

PUB. COUNTRY: United States

DOCUMENT TYPE: (COMPARATIVE STUDY)

Journal; Article; (JOURNAL ARTICLE)
(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200410

ENTRY DATE: Entered STN: 5 Jun 2003

Last Updated on STN: 17 Dec 2003 Entered Medline: 5 Oct 2004

ED Entered STN: 5 Jun 2003

Last Updated on STN: 17 Dec 2003 Entered Medline: 5 Oct 2004

AΒ For high-throughput protein structural analysis, it is indispensable to develop a reliable protein overexpression system. Although many protein overexpression systems, such as that involving Escherichia coli cells, have been developed, the number of overexpressed proteins showing the same biological activities as those of the native proteins is limited. A novel wheat germ cell-free protein synthesis system was developed recently, and most of the proteins functioning in solution were synthesized as soluble forms. This suggests the applicability of this protein synthesis method to determination of the solution structures of functional proteins. To examine this possibility, we have synthesized two (15)N-labeled proteins and obtained (1) H-(15) N HSQC spectra for them. The structural analysis of these proteins has already progressed with an E. coli overexpression system, and (1)H-(15)N HSQC spectra for biologically active proteins have already been obtained. Comparing the spectra, we have shown that proteins synthesized with a wheat germ cell-free system have the proper protein folding and enough biological activity. This is the first experimental evidence of the applicability of the wheat germ cell-free protein synthesis system to high-throughput protein structural analysis.

L7 ANSWER 28 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2003178039 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 12696166

TITLE: Recent advances in cell-free protein synthesis: application for

postgenome sciences.

AUTHOR: Endo Yaeta; Sawasaki Tatsuyayendo@eng.ehime-u.ac.jp

SOURCE: Tanpakushitsu kakusan koso. Protein, nucleic acid, enzyme,

(2003 Mar) Vol. 48, No. 4 Suppl, pp. 549-54. Ref: 12

Journal code: 0413762. ISSN: 0039-9450.

PUB. COUNTRY: Japan

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

General Review; (REVIEW)

LANGUAGE: Japanese

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200306

ENTRY DATE: Entered STN: 17 Apr 2003

Last Updated on STN: 19 Jun 2003 Entered Medline: 18 Jun 2003

ED Entered STN: 17 Apr 2003

Last Updated on STN: 19 Jun 2003 Entered Medline: 18 Jun 2003

L7 ANSWER 29 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2002669593 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 12409616

TITLE: A cell-free protein synthesis system for high-throughput

proteomics.

AUTHOR: Sawasaki Tatsuya; Ogasawara Tomio; Morishita Ryo; Endo Yaeta CORPORATE SOURCE: Department of Applied Chemistry, Faculty of Engineering, and

Venture Business Laboratory, Ehime University, Matsuyama 790-8577, Japan.

SOURCE: Proceedings of the National Academy of Sciences of the United

States of America, (2002 Nov 12) Vol. 99, No. 23, pp. 14652-7. Electronic

Publication: 2002-10-30.

Journal code: 7505876. ISSN: 0027-8424.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200301

ENTRY DATE: Entered STN: 14 Nov 2002

Last Updated on STN: 16 Jan 2003 Entered Medline: 15 Jan 2003

ED Entered STN: 14 Nov 2002

Last Updated on STN: 16 Jan 2003 Entered Medline: 15 Jan 2003

We report a cell-free system for the high-throughput synthesis and screening AΒ of gene products. The system, based on the eukaryotic translation apparatus of wheat seeds, has significant advantages over other commonly used cell-free expression systems. To maximize the yield and throughput of the system, we optimized the mRNA UTRs, designed an expression vector for large-scale protein production, and developed a new strategy to construct PCR-generated DNAs for high-throughput production of many proteins in parallel. The resulting system achieves high-yield expression and can maintain productive translation for 14 days. Additionally, in the integration of a PCR-directed system for template creation, at least 50 genes can be translated in parallel, yielding between 0.1 and 2.3 mg of protein by one person within 2 days. Assessment of correct protein folding by the products of this high-throughput protein-expression system were performed by enzymatic assays of kinases and by NMR spectroscopic analysis. The cell-free system, reported here, bypasses many of the timeconsuming cloning steps of conventional expression systems and lends itself to a robotic automation for the high-throughput expression of proteins.

L7 ANSWER 30 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2002397679 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 12097643

TITLE: An engineered Escherichia coli tyrosyl-tRNA synthetase for site-specific incorporation of an unnatural amino acid into proteins in eukaryotic translation and its application in a wheat germ cell-free system.

AUTHOR: Kiga Daisuke; Sakamoto Kensaku; Kodama Koichiro; Kigawa

Takanori; Matsuda Takayoshi; Yabuki Takashi; Shirouzu Mikako; Harada Yoko; Nakayama Hiroshi; Takio Koji; Hasegawa Yoshinori; Endo Yaeta; Hirao Ichiro; Yokoyama

Shigeyuki

CORPORATE SOURCE: RIKEN Genomic Sciences Center, 1-7-22 Suehiro-cho, Tsurumi,

Yokohama 230-0045, Japan.

SOURCE: Proceedings of the National Academy of Sciences of the United

States of America, (2002 Jul 23) Vol. 99, No. 15, pp. 9715-20. Electronic

Publication: 2002-07-03.

Journal code: 7505876. ISSN: 0027-8424.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200209

ENTRY DATE: Entered STN: 31 Jul 2002

Last Updated on STN: 5 Jan 2003 Entered Medline: 4 Sep 2002

ED Entered STN: 31 Jul 2002

Last Updated on STN: 5 Jan 2003

Entered Medline: 4 Sep 2002

AB Tyrosyl-tRNA synthetase (TyrRS) from Escherichia coli was engineered to preferentially recognize 3-iodo-L-tyrosine rather than L-tyrosine for the site-specific incorporation of 3-iodo-L-tyrosine into proteins in eukaryotic

translation systems. The wild-type TyrRS does not recognize 3-iodo-L-tyrosine, because of the bulky iodine substitution. On the basis of the reported crystal structure of Bacillus stearothermophilus TyrRS, three residues, Y37, Q179, and Q195, in the L-tyrosine-binding site were chosen for mutagenesis. Thirty-four single amino acid replacements and 16 of their combinations were screened by in vitro biochemical assays. A combination of the Y37V and Q195C mutations changed the amino acid specificity in such a way that the variant TyrRS activates 3-iodo-L-tyrosine 10-fold more efficiently than L-tyrosine. This engineered enzyme, TyrRS(V37C195), was tested for use in the wheat germ cell-free translation system, which has recently been significantly improved, and is now as productive as conventional recombinant systems. During the translation in the wheat germ system, an E. coli suppressor tRNA(Tyr) was not aminoacylated by the wheat germ enzymes, but was aminoacylated by the E. coli TyrRS(V37C195) variant with 3-iodo-l-tyrosine. After the use of the 3iodotyrosyl-tRNA in translation, the resultant uncharged tRNA could be aminoacylated again in the system. A mass spectrometric analysis of the produced protein revealed that more than 95% of the amino acids incorporated for an amber codon were iodotyrosine, whose concentration was only twice that of L-tyrosine in the translation. Therefore, the variant enzyme, 3-iodo-Ltyrosine, and the suppressor tRNA can serve as an additional set orthogonal to the 20 endogenous sets in eukaryotic in vitro translation systems.

L7 ANSWER 31 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2002355633 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 12099014

TITLE: High-throughput expression of proteins from cDNAs catalogue

from Arabidopsis in wheat germ cell-free protein synthesis system.

AUTHOR: Sawasaki Tatsuya; Seki Motoaki; Sinozaki Kazuo; Endo

Yaetasawasaki@en3.ehime-u.ac.jp

SOURCE: Tanpakushitsu kakusan koso. Protein, nucleic acid, enzyme,

(2002 Jun) Vol. 47, No. 8 Suppl, pp. 1003-8. Ref: 16

Journal code: 0413762. ISSN: 0039-9450.

PUB. COUNTRY: Japan

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

General Review; (REVIEW)

LANGUAGE: Japanese

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200208

ENTRY DATE: Entered STN: 9 Jul 2002

Last Updated on STN: 24 Aug 2002 Entered Medline: 23 Aug 2002

ED Entered STN: 9 Jul 2002

Last Updated on STN: 24 Aug 2002 Entered Medline: 23 Aug 2002

L7 ANSWER 32 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2002290082 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 12030037

TITLE: Highly efficient cell-free protein synthesis system prepared

from wheat embryos.

AUTHOR: Endo Yaeta; Sawasaki Tatsuya

CORPORATE SOURCE: Department of Applied Chemistry, Faculty of Engineering, and Venture Business Laboratory, Ehime University, 3 Bunkyo-cho, Matsuyama 790-8577. SOURCE: Seikagaku. The Journal of Japanese Biochemical Society, (2002)

Apr) Vol. 74, No. 4, pp. 326-30. Ref: 8

Journal code: 0413564. ISSN: 0037-1017.

PUB. COUNTRY: Japan

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

General Review; (REVIEW)

LANGUAGE: Japanese

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200207

ENTRY DATE: Entered STN: 28 May 2002

Last Updated on STN: 31 Jul 2002 Entered Medline: 30 Jul 2002

ED Entered STN: 28 May 2002

Last Updated on STN: 31 Jul 2002 Entered Medline: 30 Jul 2002

L7 ANSWER 33 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2002171983 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 11904191

TITLE: Ribosome display for selection of active dihydrofolate

reductase mutants using immobilized methotrexate on agarose beads.

AUTHOR: Takahashi Fumio; Ebihara Takashi; Mie Masayasu; Yanaqida

Yasuko; Endo Yaeta; Kobatake Eiry; Aizawa Masuo

CORPORATE SOURCE: Department of Biological Information, Graduate School of Bioscience and Biotechnology, Tokyo Institute of Technology, 4259 Nagatsuta,

Midoriku, 226-8501, Yokohama, Japan.

SOURCE: FEBS letters, (2002 Mar 6) Vol. 514, No. 1, pp. 106-10.

Journal code: 0155157. ISSN: 0014-5793.

PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200204

ENTRY DATE: Entered STN: 21 Mar 2002

Last Updated on STN: 30 Jul 2002 Entered Medline: 10 Apr 2002

ED Entered STN: 21 Mar 2002

Last Updated on STN: 30 Jul 2002 Entered Medline: 10 Apr 2002

AB Ribosome display was applied to the selection of an enzyme. As a model, we selected and amplified the dihydrofolate reductase (DHFR) gene by ribosome display utilizing a wheat germ cell-free protein synthesis system based on binding affinity to its substrate analog, methotrexate, immobilized on agarose beads. After three rounds of selection, the DHFR gene could be effectively selected and preferentially amplified from a small proportion in a mixture also containing competitive genes. Active enzymes were expressed and amplified and by sequence analysis, four mutants of DHFR were identified. These mutants showed as much activity as the wild-type enzyme.

L7 ANSWER 34 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2002171982 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 11904190

TITLE: A bilayer cell-free protein synthesis system for high-

throughput screening of gene products.

AUTHOR: Sawasaki Tatsuya; Hasegawa Yoshinori; Tsuchimochi Masateru;

Kamura Nami; Ogasawara Tomio; Kuroita Toshihiro; Endo Yaeta

CORPORATE SOURCE: Department of Applied Chemistry, Faculty of Engineering, The

Venture Business Laboratory, Ehime University, 790-8577, Matsuyama, Japan. SOURCE: FEBS letters, (2002 Mar 6) Vol. 514, No. 1, pp. 102-5.

Journal code: 0155157. ISSN: 0014-5793.

PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200204

ENTRY DATE: Entered STN: 21 Mar 2002

Last Updated on STN: 12 Apr 2002 Entered Medline: 10 Apr 2002

ED Entered STN: 21 Mar 2002

Last Updated on STN: 12 Apr 2002 Entered Medline: 10 Apr 2002

AB A high-throughput cell-free protein synthesis method has been described. The methodology is based on a bilayer diffusion system that enables the continuous supply of substrates, together with the continuous removal of small byproducts, through a phase between the translation mixture and substrate mixture. With the use of a multititer plate the system was functional for a prolonged time, and as a consequence yielded more than 10 times that of the similar batch-mode reaction. Combining this method with a wheat germ cell-free translation system developed by us, the system could produce a large amount of protein sufficient for carrying out functional analyses. This novel bilayer-based cell-free protein synthesis system with its simplicity, minimum time and low cost may be useful practical methodology in the post-genome era.

L7 ANSWER 35 OF 103 MEDLINE on STN

ACCESSION NUMBER: 2000105511 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 10639118

TITLE: A highly efficient and robust cell-free protein synthesis system prepared from wheat embryos: plants apparently contain a suicide system directed at ribosomes.

AUTHOR: Madin K; Sawasaki T; Ogasawara T; Endo Y

CORPORATE SOURCE: Department of Applied Chemistry, Faculty of Engineering, Ehime

University, Matsuyama 790-8577, Japan.

SOURCE: Proceedings of the National Academy of Sciences of the United

States of America, (2000 Jan 18) Vol. 97, No. 2, pp. 559-64.

Journal code: 7505876. ISSN: 0027-8424.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 200003

ENTRY DATE: Entered STN: 14 Mar 2000

Last Updated on STN: 14 Mar 2000 Entered Medline: 2 Mar 2000

ED Entered STN: 14 Mar 2000

Last Updated on STN: 14 Mar 2000 Entered Medline: 2 Mar 2000

AB Current cell-free protein synthesis systems can synthesize proteins with high speed and accuracy, but produce only a low yield because of their instability over time. Here we describe the preparation of a highly efficient but also robust cell-free system from wheat embryos. We first investigated the source of the instability of existing systems in light of endogenous ribosomeinactivating proteins and found that ribosome inactivation by tritin occurs already during extract preparation and continues during incubation for protein synthesis. Therefore, we prepared our system from extensively washed embryos that are devoid of contamination by endosperm, the source of tritin and possibly other inhibitors. In a batch system, we observed continuous translation for 4 h, and sucrose density gradient analysis showed formation of large polysomes, indicating high protein synthesis activity. When the reaction was performed in a dialysis bag, enabling the continuous supply of substrates together with the continuous removal of small byproducts, translation proceeded for >60 h, yielding 1-4 mg of enzymatically active proteins, and 0.6 mg of a 126-kDa tobacco mosaic virus protein, per milliliter of reaction volume. Our results demonstrate that plants contain endogenous

inhibitors of translation and that after their elimination the translational apparatus is very stable. This contrasts with the common belief that cell-free translation systems are inherently unstable, even fragile. Our method is useful for the preparation of large amounts of active protein as well as for the study of protein synthesis itself.

L7 ANSWER 36 OF 103 MEDLINE on STN

ACCESSION NUMBER: 1999329090 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 10400702

TITLE: Ribonuclease activity of rat liver perchloric acid-soluble

protein, a potent inhibitor of protein synthesis.

AUTHOR: Morishita R; Kawagoshi A; Sawasaki T; Madin K; Ogasawara T; Oka

T; Endo Y

CORPORATE SOURCE: Department of Applied Chemistry, Faculty of Engineering, Ehime

University, Matsuyama 790-8577, Japan.

SOURCE: The Journal of biological chemistry, (1999 Jul 16) Vol. 274,

No. 29, pp. 20688-92.

Journal code: 2985121R. ISSN: 0021-9258.

PUB. COUNTRY: United States

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

(RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199908

ENTRY DATE: Entered STN: 27 Aug 1999

Last Updated on STN: 27 Aug 1999 Entered Medline: 19 Aug 1999

ED Entered STN: 27 Aug 1999

Last Updated on STN: 27 Aug 1999 Entered Medline: 19 Aug 1999

Rat liver perchloric acid-soluble protein (L-PSP) is a potent inhibitor of AΒ cell-free protein synthesis; however, its mechanism of action is not known. Here we show that the protein is a unique ribonuclease and that this activity is responsible for the inhibition of translation. The addition of perchloric acid-soluble protein to a rabbit reticulocyte cell-free system at a concentration of 6.2 microM led to an almost complete inhibition of protein synthesis. The kinetics are unlike those of hemin-controlled inhibitor, a protein that acts at the initiation step. The inhibition appears to be due to an endoribonucleolytic activity of perchloric acid-soluble protein because L- $\operatorname{PSP}$  directly affects mRNA template activity and induces disaggregation of the reticulocyte polysomes into 80 S ribosomes, even in the presence of cycloheximide. These effects were observed with authentic as well as recombinant L-PSP. Analysis by thin-layer chromatography of [alpha-32P]UTPlabeled mRNA incubated with the protein showed production of the ribonucleoside 3'-monophosphates Ap, Gp, Up, and Cp, providing direct evidence that the protein is an endoribonuclease. When either 5'- or 3'-32P-labeled 5 S rRNA was the substrate, L-PSP cleaved phosphodiester bonds only in the single-stranded regions of the molecule.

L7 ANSWER 37 OF 103 MEDLINE on STN

ACCESSION NUMBER: 94024869 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 8211976

TITLE: Production of dihydrofolate reductase by an improved continuous

flow cell-free translation system using wheat germ extract.

AUTHOR: Endo Y; Oka T; Ogata K; Natori Y

CORPORATE SOURCE: Department of Biochemistry, Yamanashi Medical College, Japan. SOURCE: The Tokushima journal of experimental medicine, (1993 Jun) Vol. 40, No. 1-2, pp. 13-7.

Journal code: 0417356. ISSN: 0040-8875.

PUB. COUNTRY: Japan

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199311

ENTRY DATE: Entered STN: 17 Jan 1994

Last Updated on STN: 17 Jan 1994 Entered Medline: 19 Nov 1993

ED Entered STN: 17 Jan 1994

Last Updated on STN: 17 Jan 1994 Entered Medline: 19 Nov 1993

AB We have examined the characteristics of protein synthesis in an improved continuous flow cell-free translation system prepared from wheat germ extract with dihydrofolate reductase mRNA as the translated message. Continuous buffer flow and separation of the product from the reaction mixture were accomplished by the use of a modified Amicon ultrafiltration chamber as the reaction vessel. The system worked for 19 hours and produced 1.52 nmol (27.4 micrograms) of enzymatically active dihydrofolate reductase.

L7 ANSWER 38 OF 103 MEDLINE on STN

ACCESSION NUMBER: 94024866 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 8211973

TITLE: The biosynthesis of a cytotoxic protein, alpha-sarcin, in a mold Aspergillus giganteus. I. Synthesis of prepro- and pro-alpha-sarcin in vitro.

AUTHOR: Endo Y; Oka T; Tsurugi K; Natori Y

CORPORATE SOURCE: Department of Biochemistry, Yamanashi Medical College, Japan. SOURCE: The Tokushima journal of experimental medicine, (1993 Jun) Vol.

40, No. 1-2, pp. 1-6.

Journal code: 0417356. ISSN: 0040-8875.

PUB. COUNTRY: Japan

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 199311

ENTRY DATE: Entered STN: 17 Jan 1994

Last Updated on STN: 17 Jan 1994 Entered Medline: 19 Nov 1993

ED Entered STN: 17 Jan 1994

Last Updated on STN: 17 Jan 1994 Entered Medline: 19 Nov 1993

AB The biosynthesis of alpha-sarcin, a ribosome inactivating protein (16.9 KDa) was studied in a mold Aspergillus giganteus. The fungus begins to secrete alpha-sarcin after reaching a stationary phase around 50 h of culture. synthesis of alpha-sarcin was shown to be induced at the transcriptional level since the mRNA level of alpha-sarcin, titrated by immuno-precipitation with anti-alpha-sarcin antibodies of translation products in wheat germ cell-free system, was increased synchroniously with the production of the protein. The immuno-precipitates specific for alpha-sarcin contained two species of proteins of 22.5 and 18.5 KDa. The former was localized in the supernatant and the latter was segregated in the microsomes of the wheat germ system. The 22.5 KDa protein was thought to be the primary product of alpha-sarcin, although N-terminal methionine was removed, because it was the only product when the microsomes were solubilized by Triton X-100 prior to translation in the cell-free system. These results indicate that alpha-sarcin is synthesized as 22.5 KDa prepro-alpha-sarcin and is processed cotranslationary into 18.5 KDa pro-alpha-sarcin in endoplasmic reticulum as usual secretary proteins.

L7 ANSWER 39 OF 103 MEDLINE on STN

ACCESSION NUMBER: 93001007 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 1368801

TITLE: Production of an enzymatic active protein using a continuous

flow cell-free translation system.

AUTHOR: Endo Y; Otsuzuki S; Ito K; Miura K

CORPORATE SOURCE: Department of Biochemistry, Yamanashi Medical College, Japan. SOURCE: Journal of biotechnology, (1992 Sep) Vol. 25, No. 3, pp. 221-

30.

Journal code: 8411927. ISSN: 0168-1656.

PUB. COUNTRY: Netherlands

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Biotechnology

ENTRY MONTH: 199211

ENTRY DATE: Entered STN: 9 Aug 1995

Last Updated on STN: 9 Aug 1995 Entered Medline: 3 Nov 1992

ED Entered STN: 9 Aug 1995

Last Updated on STN: 9 Aug 1995 Entered Medline: 3 Nov 1992

AB We have examined the characteristics of protein synthesis in an improved continuous flow cell-free translation system prepared from wheat germ extract with dihydrofolate reductase (dhfr) mRNA as the translated message. Continuous buffer flow and separation of product from the reaction mixture were accomplished by the use of a modified Amicon ultrafiltration chamber as reaction vessel. The system produced protein for more than 20 h, and the product had an activity of dhfr comparable to that of authentic enzyme from E. coli. Analysis of RNA recovered from the filtrate supports the notion that a functionally active protein-synthesizing machinery is superorganized in a dynamic complex.

L7 ANSWER 40 OF 103 MEDLINE on STN

ACCESSION NUMBER: 88198077 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 2452154

TITLE: Characterization of a novel acidic protein of 38 kDa, A0, in yeast ribosomes which immunologically cross-reacts with the 13 kDa acidic ribosomal proteins, A1/A2.

AUTHOR: Mitsui K; Motizuki M; Endo Y; Yokota S; Tsurugi K

CORPORATE SOURCE: Department of Biochemistry, Yamanashi Medical College.

SOURCE: Journal of biochemistry, (1987 Dec) Vol. 102, No. 6, pp. 1565-

70.

Journal code: 0376600. ISSN: 0021-924X.

PUB. COUNTRY: Japan

DOCUMENT TYPE: (COMPARATIVE STUDY)

Journal; Article; (JOURNAL ARTICLE) (RESEARCH SUPPORT, NON-U.S. GOV'T)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 198806

ENTRY DATE: Entered STN: 8 Mar 1990

Last Updated on STN: 29 Jan 1996 Entered Medline: 2 Jun 1988

ED Entered STN: 8 Mar 1990

Last Updated on STN: 29 Jan 1996

Entered Medline: 2 Jun 1988

AB A new ribosomal protein of 38 kDa, named A0, was detected in yeast ribosomes on immunoblotting. The antibody used here was that against A1/A2, 13 kDa acidic ribosomal proteins which cross-reacted with A0. Although A0 and A1/A2

share common antigenic determinants, they differ in the following biochemical properties. While A1/A2 could be extracted from ribosomes with ethanol and ammonium sulfate, A0 could not. A0 gave two protein spots in a less acidic region than for A1/A2 on two-dimensional gel electrophoresis. The heterogeneity observed for A0 was ascribable to phosphorylation because one spot disappeared after treatment of the ribosomes with phosphatase. The syntheses of A0 and A1/A2 are directed by different mRNA species, as judged with a cell-free translation system, ruling out the possibility that A0 is a precursor of A1/A2. Although a mammalian ribosomal protein equivalent to A0 has been shown to be associated with 13 kDa acidic proteins in the cytoplasm, essentially no A0 was detected on immunoblotting in the yeast cytosol, while a small but detectable amount of A1/A2 was present. The possibility that A0 is a eukaryotic equivalent of L10 of Escherichia coli is discussed.

L7 ANSWER 41 OF 103 MEDLINE on STN

ACCESSION NUMBER: 82239263 MEDLINE Full-text

DOCUMENT NUMBER: PubMed ID: 7096300

TITLE: A cell-free system from ethionine-treated rat liver active in

initiation of protein synthesis.

AUTHOR: Hase M; Endo Y; Natori Y

SOURCE: Journal of biochemistry, (1982 May) Vol. 91, No. 5, pp. 1457-

65.

Journal code: 0376600. ISSN: 0021-924X.

PUB. COUNTRY: Japan

DOCUMENT TYPE: Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 198209

ENTRY DATE: Entered STN: 17 Mar 1990

Last Updated on STN: 17 Mar 1990 Entered Medline: 17 Sep 1982

ED Entered STN: 17 Mar 1990

Last Updated on STN: 17 Mar 1990 Entered Medline: 17 Sep 1982

AB A cell-free protein-synthesizing system active in initiation of translation of both endogenous mRNA and exogenous mRNA has been obtained from postmitochondrial supernatant (S-12) of the liver of ethionine-treated rats by adding reticulocyte ribosomal extract as a source of initiation factor. Formation of polysomes in the course of protein synthesis in vitro has also been demonstrated. Homogenization of the liver in the presence of 50 microM hemin stabilizes the initiation activity of S-12 fraction, which otherwise decays rapidly even at 0 degrees C. The mechanism of inhibition of protein synthesis by ethionine is discussed in view of these results.

L7 ANSWER 42 OF 103 MEDLINE on STN

ACCESSION NUMBER: 79063686 MEDLINE <u>Full-text</u>

DOCUMENT NUMBER: PubMed ID: 82483

TITLE: Protein synthesis by rat transplantable yolk sac tumor and its

relation to the cytosol levels of translatable messenger RNA's.

AUTHOR: Kaneko Y; Endo Y; Oda T

SOURCE: Cancer research, (1978 Dec) Vol. 38, No. 12, pp. 4728-33.

Journal code: 2984705R. ISSN: 0008-5472.

PUB. COUNTRY: United States
DOCUMENT TYPE: (COMPARATIVE STUDY)

(IN VITRO)

Journal; Article; (JOURNAL ARTICLE)

LANGUAGE: English

FILE SEGMENT: Priority Journals

ENTRY MONTH: 197902

ENTRY DATE: Entered STN: 14 Mar 1990

Last Updated on STN: 14 Mar 1990 Entered Medline: 12 Feb 1979

ED Entered STN: 14 Mar 1990

Last Updated on STN: 14 Mar 1990 Entered Medline: 12 Feb 1979

alpha-Fetoprotein (AFP) was shown to be the major secretory protein produced AΒ in vitro by normal rat yolk sacs. While not so active, AFP production was also detected in the transplantable tumors derived from normal volk sacs. The major secretory protein synthesized by the tumor cells had a molecular weight of 40,000 and was reactive with an anti-rat albumin antibody. The functional messenger RNA's coding for these proteins were quantitated by translation in a cell-free system derived from wheat germ followed by specific immunoprecipitation of the newly synthesized peptides. The overall template activity of the RNA prepared from the normal yolk sacs and yolk sac tumor cells was virtually identical. The cytosol RNA prepared from the normal yolk sacs was approximately 12 times more active than that from the tumor cells in directing the synthesis of AFP. The presence of the cytosol RNA prepared from the tumor cells was required for the synthesis of proteins immunoprecipitable with the antialbumin antibody. These results suggest that the changes in AFP and albumin synthesis can be accounted for by a corresponding change in the levels of functional messenger RNA's coding for these proteins.

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ACCESSION NUMBER: 2008:243702 BIOSIS Full-text

DOCUMENT NUMBER: PREV200800243303

TITLE: Expression of malaria vaccine candidates using a wheat germ

cell-free protein synthesis system without codon optimisation.

AUTHOR(S): Tsuboi, Takafumi [Reprint Author]; Takeo, Satoru; Iriko, Hideyuki; Jin, Ling; Tsuchimochi, Masateru; Matsuda, Shusaku; Han, Eun-Taek; Otsuki, Hitoshi; Kaneko, Osamu; Sattabongkot, Jetsumon; Udomsangpetch, Rachanee; Sawasaki.

Tatsuya; Torii, Motomi; Endo, Yaeta

CORPORATE SOURCE: Ehime Univ, Cell Free Sci and Technol Res Ctr, Matsuyama,

Ehime, Japan

SOURCE: International Journal for Parasitology, (JAN 2008) Vol. 38, No.

Suppl. 1, pp. S77.

Meeting Info.: 3rd Molecular Approaches to Malaria Meeting (MAM 2008). Lorne, AUSTRALIA. February 03 -07, 2008. BioMalPar; Boehringer Ingelheim Foods; Burroughs Wellcome Fund; Fdn Natl Inst Hlth; PATH Malaria Vaccine Initiative;

Walter & Eliza Hall Inst Med Res; Wellcome Trust; ARC/NHMRC Net Parasitol; Australian Soc Biochem & Molecular Biol; Lorne Protein Conf; GlaxoSmithKline.

CODEN: IJPYBT. ISSN: 0020-7519.

DOCUMENT TYPE: Conference; (Meeting)

Conference; (Meeting Poster)

LANGUAGE: English

ENTRY DATE: Entered STN: 2 Apr 2008

Last Updated on STN: 2 Apr 2008

ED Entered STN: 2 Apr 2008

Last Updated on STN: 2 Apr 2008

L7 ANSWER 44 OF 103 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN

ACCESSION NUMBER: 2007:605234 BIOSIS Full-text

DOCUMENT NUMBER: PREV200700606893

TITLE: Methods for high-throughput materialization of genetic

information based on wheat germ cell-free expression system.

AUTHOR(S): Sawasaki, Tatsuya [Reprint Author]; Morishita, Ryo; Gouda,

Mudeppa D.; Endo, Yaeta

CORPORATE SOURCE: Ehime Univ, Cell Free Sci and Technol Res Ctr, Matsuyama, Ehime

790, Japan

SOURCE: Grandi, G [Editor]. Methods in Molecular Biology, (2007) pp.

95-106. Methods in Molecular Biology.

Publisher: HUMANA PRESS INC, 999 RIVERVIEW DR, STE 208, TOTOWA,

NJ 07512-1165 USA. Series: METHODS IN MOLECULAR BIOLOGY.

ISSN: 1064-3745. ISBN: 978-1-58829-558-3(H).

DOCUMENT TYPE: Book; (Book Chapter)

LANGUAGE: English

ENTRY DATE: Entered STN: 6 Dec 2007

Last Updated on STN: 6 Dec 2007

ED Entered STN: 6 Dec 2007

Last Updated on STN: 6 Dec 2007

AB Among the cell-free protein synthesis systems, the wheat germ-based translation system has significant advantages for the high-throughput production of eukaryotic multidomain proteins in folded state. Here, we describe protocols for this cell-free expression system.

L7 ANSWER 45 OF 103 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN

ACCESSION NUMBER: 2007:442841 BIOSIS Full-text

DOCUMENT NUMBER: PREV200700439848

TITLE: Preparation containing cell extracts for cell-free protein

synthesis and means for synthesizing protein using the preparation.

AUTHOR(S): Anonymous; Endo, Yaeta [Inventor]; Nishikawa, Shiqemichi

[Inventor]

CORPORATE SOURCE: Matsuyama, Japan

ASSIGNEE: CellFree Sciences Co Ltd

PATENT INFORMATION: US 07235382 20070626

SOURCE: Official Gazette of the United States Patent and Trademark

Office Patents, (JUN 26 2007)

CODEN: OGUPE7. ISSN: 0098-1133.

DOCUMENT TYPE: Patent LANGUAGE: English

ENTRY DATE: Entered STN: 15 Aug 2007

Last Updated on STN: 15 Aug 2007

ED Entered STN: 15 Aug 2007

Last Updated on STN: 15 Aug 2007

Disclosed are a preparation containing cell extracts for cell-free protein synthesis, prepared by excluding from a living organism a system, participating to inhibiting of self protein synthesis reaction, an apparatus for cell-free protein synthesis reaction equipped with a reaction tank for cell-free protein synthesis, and a kit for use therefor; the preparation can be stored at room temperature and prepared as a preparation in a state where biological functions of the cell extracts are maintained, and further, disclosed is methods for cell-free protein synthesis comprising providing cell extracts from which an inhibitor for self protein synthesis reaction is substantially excluded, having introduced therein treatment selected from supplement, storage, exchange or discharge with respect to an element selected from at least mRNA serving as a template for synthesis reaction, an energy reproduction system enzyme, a substrate, and an energy source.

L7 ANSWER 46 OF 103 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN

ACCESSION NUMBER: 2006:669281 BIOSIS Full-text

DOCUMENT NUMBER: PREV200600682572

TITLE: General means of labeling protein by using wheat embryo cell-

free protein synthesis system.

AUTHOR(S): Anonymous; Endo, Yaeta [Inventor]; Kumar, Penmetcha [Inventor];

Nishikawa, Shigemichi [Inventor] CORPORATE SOURCE: Matsuyama, Japan

ASSIGNEE: CellFree Sciences Co Ltd

PATENT INFORMATION: US 07074595 20060711

SOURCE: Official Gazette of the United States Patent and Trademark

Office Patents, (JUL 11 2006)

CODEN: OGUPE7. ISSN: 0098-1133.

DOCUMENT TYPE: Patent LANGUAGE: English

ENTRY DATE: Entered STN: 6 Dec 2006

Last Updated on STN: 6 Dec 2006

ED Entered STN: 6 Dec 2006

Last Updated on STN: 6 Dec 2006

AB Utilizing a what embryo cell-free protein synthesis system, there are provided a process for the production of selenomethionine-labeled protein, characterized in that, methionine in a wheat embryo extract for a cell-free protein synthesis obtained by a complete removal of endosperm contaminated is changed to selenomethionine and a cell-free protein synthesis is carried out using a reaction solution composition for protein synthesis containing selenomethionine instead of methionine under a batch condition or a dialysis condition and also the said protein produced as such. There are further provided a process for the production of heavy hydrogen-labeled protein using the same means and also the said protein produced as such.

L7 ANSWER 47 OF 103 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN

ACCESSION NUMBER: 2006:575286 BIOSIS Full-text

DOCUMENT NUMBER: PREV200600571389

TITLE: Covalent circularization of exogenous RNA during incubation

with a wheat embryo cell extract.

AUTHOR(S): Makino, Shin-ichi; Sawasaki, Tatsuya; Tozawa, Yuzuru; Endo,

Yaeta; Takai, Kazuyuki [Reprint Author]

CORPORATE SOURCE: Ehime Univ, Venture Business Lab, 3 Bunkyo Cho, Matsuyama,

Ehime 7908577, Japan

takai@eng.ehime-u.ac.jp

SOURCE: Biochemical and Biophysical Research Communications, (SEP 8

2006) Vol. 347, No. 4, pp. 1080-1087.

CODEN: BBRCA9. ISSN: 0006-291X.

DOCUMENT TYPE: Article LANGUAGE: English

ENTRY DATE: Entered STN: 1 Nov 2006

Last Updated on STN: 1 Nov 2006

ED Entered STN: 1 Nov 2006

Last Updated on STN: 1 Nov 2006

AB Cell extracts from wheat embryos have been widely used for mRNA-directed protein production. Here, we found that a significant fraction of exogenous linear RNAs are circularized in a wheat embryo extract. The circularization was seen only in uncapped RNAs. The amount of the circular species reached around 1% of the initial RNA and increased along with an increase in the initial concentration more than proportionally. The circular RNAs were stable but unable to be translated in the extract. The circularization was competitively inhibited in the presence of a known substrate of a wheat embryo RNA ligase. Thus, we cloned the RNA ligase cDNAs. Three isoform sequences were homologous to the other plant RNA ligases. An addition of a cell-free synthesized wheat RNA ligase abolished the inhibition, which indicates a participation of its activity in the circularization. A possible role in RNA metabolism, RNA silencing in particular, is discussed. (c) 2006 Elsevier Inc. All rights reserved.

L7 ANSWER 48 OF 103 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN

ACCESSION NUMBER: 2006:212186 BIOSIS Full-text

DOCUMENT NUMBER: PREV200600215350

TITLE: Preparation containing cell extracts for synthesizing cell-free

protein and means for synthesizing cell-free protein.

AUTHOR(S): Endo, Yaeta [Inventor]; Nishikawa, Shigemichi [Inventor]

CORPORATE SOURCE: Matsuyama, Japan

ASSIGNEE: CellFree Sciences Co., Ltd.

PATENT INFORMATION: US 06905843 20050614

SOURCE: Official Gazette of the United States Patent and Trademark

Office Patents, (JUN 14 2005)

CODEN: OGUPE7. ISSN: 0098-1133.

DOCUMENT TYPE: Patent LANGUAGE: English

ENTRY DATE: Entered STN: 29 Mar 2006

Last Updated on STN: 29 Mar 2006

ED Entered STN: 29 Mar 2006

Last Updated on STN: 29 Mar 2006

Disclosed are a preparation containing cell extracts for cell-free protein synthesis, prepared by excluding from a living organism a system, participating to inhibiting of self protein synthesis reaction, an apparatus for cell-free protein synthesis reaction equipped with a reaction tank for cell-free protein synthesis, and a kit for use therefor; the preparation can be stored at room temperature and prepared as a preparation in a state where biological functions of the cell extracts are maintained and further, disclosed is means for cell-free protein synthesis comprising cell extracts from which an inhibitor for self protein synthesis reaction is substantially excluded, having introduced therein treatment selected from supplement, storage, exchange or discharge with respect to an element selected from at least mRNA serving as a template for synthesis reaction, an energy reproduction system enzyme, a substrate, and an energy source.

L7 ANSWER 49 OF 103 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN

ACCESSION NUMBER: 2006:133738 BIOSIS <u>Full-text</u>

DOCUMENT NUMBER: PREV200600143348

TITLE: Methods of synthesizing cell-free protein.

AUTHOR(S): Endo, Yaeta [Inventor]; Sawasaki, Tatsuya [Inventor];

Ogasawara, Tomio [Inventor]

CORPORATE SOURCE: Ehime 791-8016, Japan

ASSIGNEE: Yaeta Endo

PATENT INFORMATION: US 06869774 20050322

SOURCE: Official Gazette of the United States Patent and Trademark

Office Patents, (MAR 22 2005)

CODEN: OGUPE7. ISSN: 0098-1133.

DOCUMENT TYPE: Patent LANGUAGE: English

ENTRY DATE: Entered STN: 22 Feb 2006

Last Updated on STN: 22 Feb 2006

ED Entered STN: 22 Feb 2006

Last Updated on STN: 22 Feb 2006

AB One embodiment of the present invention is a diffusion continuous batch cell-free protein-synthesis method characterized simultaneously by continuously supplying substrate and energy source molecules in the supply phase to the reaction phase by the free diffusion via interface between both phases and by transferring by-products formed in the reaction phase by enhancing the efficiency of the synthesis reaction by prolonging the reaction lifetime by directly contacting a synthesis reaction mixture (reaction phase) containing a biological extract with a substrate- and energy source-supplying solution

(supply phase) without using barrier such as semi-permeable membrane or ultrafiltration membrane in a general cell-free protein-synthesis reaction means. Another embodiment of the present invention is a dilution batch cell-free protein synthesis method characterized by enhancing the efficiency of the protein synthesis by prolonging the reaction lifetime by adding a diluting solution to the reaction mixture after pre-incubating the reaction mixture in a cell- free protein-synthesis reaction means using a wheat-embryo extract. Another embodiment of the present invention is a method characterized by enhancing the efficiency of the synthesis reaction simultaneously by resupplying substrate and energy sources necessary for the protein synthesis (e.g., amino acids, ATP, GTP, creatine phosphate) to the reaction mixture using a gel filtration column and/or semipermeable membrane and by discontinuously removing by-products formed during the reaction after the synthesis reaction stops in the batch cell-free protein synthesis method.

L7 ANSWER 50 OF 103 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN

ACCESSION NUMBER: 2002:158200 BIOSIS Full-text

DOCUMENT NUMBER: PREV200200158200

TITLE: Recent advances in the cell-free protein synthesis system.

AUTHOR(S): Sawasaki, Tatsuya [Reprint author]; Endo, Yaeta [Reprint

author]

CORPORATE SOURCE: Applied Chemistry, Ehime University, Matsuyama, Japan SOURCE: Molecular Biology of the Cell, (Nov, 2001) Vol. 12, No.

Supplement, pp. 392a. print.

Meeting Info.: 41st Annual Meeting of the American Society for Cell Biology. Washington DC, USA. December 08-12, 2001. American Society for Cell Biology.

CODEN: MBCEEV. ISSN: 1059-1524.

DOCUMENT TYPE: Conference; (Meeting)

Conference; Abstract; (Meeting Abstract)

LANGUAGE: English

ENTRY DATE: Entered STN: 21 Feb 2002

Last Updated on STN: 26 Feb 2002

ED Entered STN: 21 Feb 2002

Last Updated on STN: 26 Feb 2002

L7 ANSWER 51 OF 103 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN

ACCESSION NUMBER: 1979:230748 BIOSIS <u>Full-text</u>
DOCUMENT NUMBER: PREV197968033252; BA68:33252

TITLE: DIRECT ASSOCIATION OF MESSENGER RNA CONTAINING RIBO NUCLEO PROTEIN PARTICLES WITH MEMBRANES OF THE ENDOPLASMIC RETICULUM IN ETHIONINE TREATED

RAT LIVER.

AUTHOR(S): ENDO Y [Reprint author]; NATORI Y

CORPORATE SOURCE: DEP NUTR CHEM, TOKUSHIMA UNIV SCH MED, TOKUSHIMA 770, JPN

SOURCE: Biochimica et Biophysica Acta, (1979) Vol. 562, No. 2, pp. 281-

291.

CODEN: BBACAQ. ISSN: 0006-3002.

DOCUMENT TYPE: Article
FILE SEGMENT: BA
LANGUAGE: ENGLISH

AB The administration of ethionine to female rats causes breakdown of hepatic polysomes. The fate of the mRNA molecules after polysome breakdown was investigated by measuring the amount of poly(A)-containing mRNA in membranous and non-membranous fractions obtained from the cytoplasm of ethionine-treated rat liver. The amount of poly(A)-containing mRNA in the membrane fraction of ethionine-treated liver was the same as that of normal liver. When poly(A)-containing mRNAs from various fractions were translated in a wheat germ system and the products were isolated by immunoprecipitation, the albumin-specific mRNA was found exclusively in the membrane fraction of both normal and

ethionine-treated livers. The membrane-bound mRNA in ethionine-treated liver, selectively labeled with [14C]orotate, was banded in CsCl gradient centrifugation at 1.42 g/ml which corresponds to the previously reported mRNA-containing ribonucleoprotein particles. Even after the polysome disaggregation by ethionine, most of the mRNA of membrane-bound polysomes apparently remains attached to the endoplasmic reticulum membranes independently of ribosomes and the nascent polypeptide chains.

L7 ANSWER 52 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2008:527505 CAPLUS Full-text

TITLE: A set of ligation-independent in vitro translation vectors

for eukaryotic protein production

AUTHOR(S): Bardoczy, Viola; Geczi, Viktoria; Sawasaki, Tatsuya; Endo,

Yaeta; Meszaros, Tamas

CORPORATE SOURCE: Department of Applied Biotechnology and Food Science,

Budapest University of Technology and Economics, Budapest, Hung. SOURCE: BMC Biotechnology (2008), 8, No pp. given

CODEN: BBMIE6; ISSN: 1472-6750

URL: http://www.biomedcentral.com/content/pdf/1472-6750-8-

32.pdf

PUBLISHER: BioMed Central Ltd.

DOCUMENT TYPE: Journal; (online computer file)

LANGUAGE: English
ED Entered STN: 01 May 2008

Background: The last decade has brought the renaissance of protein studies and AΒ accelerated the development of high-throughput methods in all aspects of proteomics. Presently, most protein synthesis systems exploit the capacity of living cells to translate proteins, but their application is limited by several factors. A more flexible alternative protein production method is the cell-free in vitro protein translation. Currently available in vitro translation systems are suitable for high-throughput robotic protein production, fulfilling the requirements of proteomics studies. Wheat germ extract based in vitro translation system is likely the most promising method, since numerous eukaryotic proteins can be cost-efficiently synthesized in their native folded form. Although currently available vectors for wheat embryo in vitro translation systems ensure high productivity, they do not meet the requirements of state-of-the-art proteomics. Target genes have to be inserted using restriction endonucleases and the plasmids do not encode cleavable affinity purification tags. Results: We designed four ligation independent cloning (LIC) vectors for wheat germ extract based in vitro protein translation. In these constructs, the RNA transcription is driven by T7 or SP6 phage polymerase and two TEV protease cleavable affinity tags can be added to aid protein purifn. To evaluate our improved vectors, a plant mitogen activated protein kinase was cloned in all four constructs. Purifn. of this eukaryotic protein kinase demonstrated that all constructs functioned as intended: insertion of PCR fragment by LIC worked efficiently, affinity purifn. of translated proteins by GST-Sepharose or MagneHis particles resulted in high purity kinase, and the affinity tags could efficiently be removed under different reaction conditions. Furthermore, high in vitro kinase activity testified of proper folding of the purified protein. Conclusion: Four newly designed in vitro translation vectors have been constructed which allow fast and parallel cloning and protein purifn., thus representing useful mol. tools for high-throughput prodn. of eukaryotic proteins.

L7 ANSWER 53 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2008:420693 CAPLUS Full-text

TITLE: Hetero subunits assembly study of RNA modification enzyme

by wheat germ cell-free translation system

AUTHOR(S): Matsumoto, Keisuke; Abe, Masato; Takano, Yoshitaka;

Takayanagi, Naoyuki; Endo, Yaeta; Hori, Hiroyuki

CORPORATE SOURCE: Graduate School of Materials Science and Biotechnology,

Ehime University, Japan

SOURCE: IEEE International Symposium on Micro-NanoMechatronics and

Human Science, Nagoya, Japan, Nov. 6-8, 2006 (2006), 328-333. Institute of

Electrical and Electronics Engineers: New York, N. Y.

CODEN: 69KOAR; ISBN: 1-4244-0717-6

DOCUMENT TYPE: Conference LANGUAGE: English ED Entered STN: 04 Apr 2008

In the living cells, many kinds of modified nucleosides exist in various RNA AΒ species. These modified nucleosides are generated by specific RNA modification enzymes. In almost cases, RNA modification enzyme is composed by one single subunit or homo-subunits. However, recent studies have revealed that eukaryote tRNA (m7G46) methyltransferases are exceptionally constituted by hetero-subunits (Trm8/Trm82 in yeast; METTL1/WDR4 in human). This enzyme catalyzes the methyl-transfer from S-adenosyl-L-methionine to the N7 atom of the semi-conserved quanosine at position 46 in the extra-loop of tRNA. To clarify the functions of two subunits, we employed wheat germ cell-free translation system. When the Trm8 or Trm82 subunit alone was synthesized, methyl-transfer activity was not detectable. In contrast, when both Trm8 and Trm82 subunits were synthesized together, tRNA methyltransferase activity was clearly detected. Furthermore, we mixed two subunits after the synthesis, however formation of the active hetero-dimer was not observed. These results demonstrated that the formation of the active Trm8 and Trm82 hetero-dimer requires the subunit-subunit interaction during the protein synthesis.

L7 ANSWER 54 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2008:98117 CAPLUS Full-text

DOCUMENT NUMBER: 148:489844

TITLE: The wheat germ cell-free protein synthesis system

AUTHOR(S): Sawasaki, Tatsuya; Endo, Yaeta

CORPORATE SOURCE: Cell-Free Science and Technology Research Center, Ehime

University, Matsuyama, 790-8577, Japan

SOURCE: Cell-Free Protein Synthesis (2008), 111-139. Editor(s): Spirin, Alexander S.; Swartz, James R. Wiley-VCH Verlag GmbH & Co. KGaA: Weinheim, Germany.

CODEN: 69KIQP; ISBN: 978-3-527-31649-6

DOCUMENT TYPE: Conference LANGUAGE: English ED Entered STN: 25 Jan 2008

AB Among cell-free protein synthesis systems, the wheat germ-based translation system is of special interest for its eukaryotic nature: it has significant advantages for the high-throughput production of eukaryotic multi-domain proteins in the folded state. Here the authors describe how this highly efficient cell-free expression system is built and review its application to today's functional and structural biol.

L7 ANSWER 55 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2007:871114 CAPLUS <u>Full-text</u>

DOCUMENT NUMBER: 147:253468

TITLE: Biotinylated protein preparation method, and detection

method using biotinylated protein

INVENTOR(S): Endo, Yaeta; Sawasaki, Tatsuya; Matsubara, Yuko

PATENT ASSIGNEE(S): Cellfree Sciences Co., Ltd., Japan

SOURCE: Jpn. Kokai Tokkyo Koho, 20pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT:

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
				-	
JP 2007199047	A	20070809	JP 2006-182785		20060630
US 20070190579	A1	20070816	US 2006-643737		20061221
PRIORITY APPLN. INFO.:			JP 2005-377840	Α	20051228
			JP 2006-182785	Α	20060630

ED Entered STN: 09 Aug 2007

AB A biotinylated protein preparation method is provided, which enables to prepare a biotin-tagged protein to be used in a detection method (e.g., ALPHA, SPR, FCS, FIDA, ELISA, DELFIA, SPA, FRET, BRET, EFC, FP) for a substance capable of interacting with the protein without requiring a free biotin removal process. In this method for preparing a biotinylated protein, a protein biotinylation is achieved with a biotin derivative of a remarkably lower concentration than the conventional biotinylation operation by carrying out a biotinylation using a biotinylation enzyme and the biotin derivative during or after a protein synthesis using a cell-free protein synthesis system, especially, a wheat germ cell-free protein synthesis system.

L7 ANSWER 56 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2007:867314 CAPLUS <u>Full-text</u>

DOCUMENT NUMBER: 147:184148

TITLE: Protein-binding carrier for efficient recovery of protein

in cell-free translation

INVENTOR(S): Endo, Yaeta

PATENT ASSIGNEE(S): Cellfree Sciences Co., Ltd., Japan

SOURCE: Jpn. Kokai Tokkyo Koho, 18pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2007195545	A	20070809	JP 2006-350351	20061226
PRIORITY APPLN. INFO.:			JP 2005-372957 A	20051226

ED Entered STN: 09 Aug 2007

AB The protein-binding carrier is selected from specific protein-binding substance-immobilized bead, magnetic bead, ion-exchanger, and affinity resin. The protein binding carrier is useful for prevention of clogging of membrane of protein in com. manufacture of protein by cell-free translation. It is also allowing repeated use of the cell-free translation system. Also, the protein thus obtain retain original structure. Recovery of green fluorescence protein which is tagged with glutathione-S transferase in wheat germ cell-free translation system with glutathione magnetic bead was shown.

L7 ANSWER 57 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2007:838548 CAPLUS <u>Full-text</u>

DOCUMENT NUMBER: 147:210145

TITLE: Preparation of antigens from Rubella virus using wheat

 $\operatorname{\mathsf{germ}}$  protein synthesis system and their use for evaluation of the risk of

congenital rubella syndrome

INVENTOR(S): Endo, Yaeta; Tsuboi, Takafumi; Okuyama, Masaaki; Oseto,

Mitsuaki

PATENT ASSIGNEE(S): Cellfree Sciences Co., Ltd., Japan

Jpn. Kokai Tokkyo Koho, 20pp. SOURCE:

CODEN: JKXXAF

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO. KIND DATE APPLICATION NO. DATE JP 2007191477 A 20070802 JP 2006-346072 20061222 RITY APPLN. INFO.: JP 2005-370320 A 20051222 PRIORITY APPLN. INFO.:

ED Entered STN: 02 Aug 2007

This invention provides a process of preparation of antigens using wheat germ AB protein synthesis system in which the endosperm and low mol. protein synthesis inhibitors were eliminated. The proteins were derived from Rubella virus El and E2 proteins, and the amino acid sequences of these proteins were disclosed. The proteins were able to cross react to the antibodies in serum of mouse immunized with the antigents. The antigens provided in this invention can be used for preparation of test kit for evaluation of the risk of congenital rubella syndrome in females.

ANSWER 58 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2007:677102 CAPLUS <u>Full-text</u>
DOCUMENT NUMBER: 148:373346

TITLE: Production of protein for nucle

TITLE: Production of protein for nuclear magnetic resonance study

using the wheat germ cell-free system

AUTHOR(S): Kohno, Toshiyuki; Endo, Yaeta
CORPORATE SOURCE: Molecular Structure Research Group, Mitsubishi Kagaku

Institute of Life Sciences (MITILS), Tokyo, Japan

Methods in Molecular Biology (Totowa, NJ, United States) (2007), 375(In Vitro Transcription and Translation Protocols (2nd Edition)), 257-

2.72

CODEN: MMBIED; ISSN: 1064-3745

PUBLISHER: Humana Press Inc.

Journal DOCUMENT TYPE: LANGUAGE: English ED Entered STN: 22 Jun 2007

NMR methods have been developed to determine the three-dimensional structures AΒ of proteins, to estimate protein folding, and to discover high-affinity ligands for proteins. However, one of the difficulties encountered in the application of such NMR methods to proteins is that the authors should obtain milligram quantities of 15N and/or 13C-labeled pure proteins of interest. Here, the authors describe the method to produce proteins for NMR expts. using the improved wheat germ cell-free system, which exhibits several attractive features for high-throughput NMR study of proteins.

REFERENCE COUNT: 12 THERE ARE 12 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

ANSWER 59 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2007:496711 CAPLUS <u>Full-text</u>

DOCUMENT NUMBER: 147:270844

TITLE: History and use of protein expression system

Endo, Yaeta AUTHOR(S):

Ehime Univ., Japan CORPORATE SOURCE:

Baiotekunoroji Janaru (2007), 7(2), 230-233 SOURCE:

CODEN: BJAAA8; ISSN: 1349-7448

PUBLISHER: Yodosha DOCUMENT TYPE: Journal; General Review

LANGUAGE: Japanese ED Entered STN: 08 May 2007

AB A review discussed brief history of the development of the technologies for recombinant protein expression systems. Features of the novel protein expression systems recently developed were overviewed. Such expression systems included the system using cold shock vector for improving protein misfolding from TakaraBio Ltd., and the systems to produce proteins with more native characteristics by using "Superwarm" insect cell expression system from Katakura Kogyo Ltd. or by using mammalian cell systems (Invitrogen). Proceeding of the protein expression technol. by using cell free protein synthesis systems was also discussed. Advantages of the cell free system were discussed by comparing E. coli, rabbit reticulocyte and wheat germ lysate systems.

L7 ANSWER 60 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2007:428031 CAPLUS <u>Full-text</u>

DOCUMENT NUMBER: 146:375315

TITLE: Freeze-dried template for cell-free protein synthesis and

laboratory application

INVENTOR(S): Endo, Yaeta; Sawasaki, Tatsuya; Tanaka, Michihiro;

Morishita, Akira; Saeki, Mihoro

PATENT ASSIGNEE(S): Cellfree Sciences Co., Ltd., Japan

SOURCE: Jpn. Kokai Tokkyo Koho, 27pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2007097438	A	20070419	JP 2005-288613	20050930
PRIORITY APPLN. INFO.:			JP 2005-288613	20050930

ED Entered STN: 19 Apr 2007

AB The freeze-dried template is nucleotide sequence for visible proteins selected from green fluorescent protein, blue fluorescent protein, etc. The RNA polymerase and phosphotransferase such as creatine kinase used in the cell - free protein synthesis do not contain animal and microbial contaminants. With proper solns., the freeze-dried template-containing cell-free protein synthesis system may be easily used, and protein synthesis monitored by the presence of visible proteins. Also, the cell-free protein synthesis system such as wheat germ extract does not use expansive RNase inhibitors.

L7 ANSWER 61 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2007:391722 CAPLUS <u>Full-text</u>

DOCUMENT NUMBER: 146:498906

TITLE: Wheat germ cell-free protein synthesis system: an

application to malaria vaccine research

AUTHOR(S): Tsuboi, Takafumi; Takeo, Satoru; Iriko, Hideyuki; Kaneko,

Osamu; Torii, Motomi; Endo, Yaeta

CORPORATE SOURCE: Cell-Free Science and Technology Research Center, Ehime

University, Japan

SOURCE: Ehime Igaku (2007), 26(1), 8-11 CODEN: EHIGEL; ISSN: 0286-3677

PUBLISHER: Ehime Igakkai

DOCUMENT TYPE: Journal; General Review

LANGUAGE: Japanese

ED Entered STN: 09 Apr 2007

AB A review discusses application of wheat germ cell-free protein system in indentification of antigens for malaria vaccine.

L7 ANSWER 62 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2007:185804 CAPLUS <u>Full-text</u>

DOCUMENT NUMBER: 146:246573

TITLE: Cell-free protein production and its application to post-

genomic biotechnology

AUTHOR(S): Endo, Yaeta

CORPORATE SOURCE: Cell-Free Sci. Technol. Res. Cent., Ehime University,

Matsuyama, 790-8577, Japan

SOURCE: Baiosaiensu to Indasutori (2007), 65(1), 11-17

CODEN: BIDSE6; ISSN: 0914-8981

PUBLISHER: Baioindasutori Kyokai DOCUMENT TYPE: Journal; General Review

LANGUAGE: Japanese ED Entered STN: 20 Feb 2007

AB A review on (1) development of highly effective cell-free protein synthesis system using wheat embryos, (2) improvement of the system by the optimization of the mRNA UTRs, expression vectors, PCR, and translation conditions, (3) development of automated protein synthesis apps., and (4) application of wheat-embryo cell-free protein synthesis system.

L7 ANSWER 63 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2007:49814 CAPLUS <u>Full-text</u>

DOCUMENT NUMBER: 146:396998

TITLE: Cell-free production of functional Plasmodium falciparum

dihydrofolate reductase-thymidylate synthase

AUTHOR(S): Mudeppa, Devaraja G.; Pang, Cullen K. T.; Tsuboi,

Takafumi; Endo, Yaeta; Buckner, Fredrick S.; Varani, Gabriele; Rathod, Pradipsinh

Κ.

CORPORATE SOURCE: Department of Chemistry, University of Washington,

Seattle, WA, 98195-1700, USA

SOURCE: Molecular & Biochemical Parasitology (2007), 151(2), 216-

219

CODEN: MBIPDP; ISSN: 0166-6851

PUBLISHER: Elsevier Ltd.

DOCUMENT TYPE: Journal LANGUAGE: English ED Entered STN: 16 Jan 2007

Expression of Plasmodium falciparum dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) in a cell-free system is described. Using a SP6 polymerase promoter, the PfDHFR-TS was expressed in the wheat germ system for protein expression. The expressed PfDHFR-TS was purified by dialysis based protocol and the purified protein was characterized. The results show that the amount of the PfDHFR-TS produced in this system was about 100µg of soluble product per mL of the translation reaction thus making this cell free system superior to other systems. The activity of the PfDHFR-TS was determined to be 30nmol/min per mL of the wheat germ extract, and based on the activity of 120/min, this translates into the amts. produced by 4500 mL of the

P.falciparum culture. Enzyme kinetics for PfDHFR-TS was also studied. REFERENCE COUNT: 17 THERE ARE 17 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 64 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2007:14862 CAPLUS Full-text DOCUMENT NUMBER: 146:95367

TITLE: Novel use of DNA-binding proteins as fusion protein tags INVENTOR(S): Endo, Yaeta; Sawasaki, Tatsuya; Kamura, Nami; Matsubara,

Yuko

PATENT ASSIGNEE(S): Cellfree Sciences Co., Ltd., Japan

SOURCE: PCT Int. Appl., 51pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

	PATENT NO.		KII	KIND		DATE 2		APPLICATION NO.					I	DATE						
					_					_										
	WO	200	7000	972		A.	L	200	7010	4	WO 2	2006-	-JP3	1271	5	,	2006	0626		
		W:	ΑE	, AG	, AL,	AM,	AT	, AU	, AZ	, BA	, BB	, BG,	BR,	, BW,	, BY	, BZ	, CA	, CH	, CN	,
CO,	CR,	CU,	CZ,	DE,	DK,	DM,	DZ,	EC,	EE,	EG,	ES,	FI,	GB,	GD,	GE,	GH,	GM,	HN,	HR,	HU,
ID,	IL,	IN,	IS,	JP,	ΚE,	KG,	KM,	KN,	KP,	KR,	KΖ,	LA,	LC,	LK,	LR,	LS,	LT,	LU,	LV,	LY,
			MA	, MD	, MG,	MK,	MN	, MW	, MX	, MZ	, NA	, NG,	NI,	, NO,	NZ,	, OM,	, PG	, PH	, PL	,
PT,	RO,	RS,	RU,	SC,	SD,	SE,	SG,	SK,	SL,	SM,	SY,	ΤJ,	TM,	TN,	TR,	TT,	TZ,	UA,	UG,	US,
UZ,	VC,	VN,	ZA,	ZM,	ZW															
		RW	: AT	, BE	, BG,	CH,	CY,	, CZ,	, DE	, DK	EE,	, ES,	FI,	, FR,	, GB	, GR	, HU	, IE	, IS	,
ΙΤ,	LT,	LU,	LV,	MC,	NL,	PL,	PT,	RO,	SE,	SI,	SK,	TR,	BF,	ВJ,	CF,	CG,	CI,	CM,	GA,	GN,
GQ,	GW,	ML,	MR,	NE,	SN,	TD,	ΤG,	BW,	GH,	GM,	ΚE,	LS,	MW,	MZ,	NA,	SD,	SL,	SZ,	TZ,	UG,
			ZM	, ZW	, AM,	AZ,	BY	, KG	, KZ	, MD	, RU	, TJ,	, TM							
PRI	ORIT	Y AP	PLN.	INF	o.:						JP 2	2005-	-187	468		A 2	2005	0627		
											JP 2	2005-	-286	956		Α 2	2005	0930		

ED Entered STN: 05 Jan 2007

Disclosed is a novel use of DNA-binding proteins as fusion protein tags for AB efficient labeling, detection or purification of the target proteins. DNAbinding domains of the binding proteins, more specifically transcription factors or DNA-binding nuclear receptors are used as tags. Three amino acid sequences that specify the DNA-binding proteins are claimed. Tag-moieties is immobilized on carriers by using the interaction of avidin/biotin, maltosebinding protein/maltose, G-protein/guanine nucleotide, DNA-binding protein/DNA, antigen (epitope)/antibody, calmodulin-binding peptide/calmodulin, ATP-binding protein/ATP, or estradiol receptor/estradiol. The fusion protein containing the DNA-binding domain can be captured and purified by using affinity matrixes with DNAs containing corresponding specific sequences. Tags have amino acid sequences sensitive to endopeptidases for proteolysis to recover the target protein. The tagged fusion proteins are designed to be prepared in the cell free protein synthesis system using wheat germ lysate. The tagged fusion proteins are expressed in individual microwells and they are immobilized on carrier matrixes. Microwells containing one or two fusion proteins form protein microarrays (protein chips).

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 65 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2006:494180 CAPLUS  $\underline{\text{Full-text}}$ 

DOCUMENT NUMBER: 145:6561

TITLE: Call-free protein synthesis systems for detecting antibody

and screening specific protein or antigen

INVENTOR(S): Endo, Yaeta; Sawasaki, Tatsuya; Ogasawara, Tomio;

Tsuchimochi, Masateru; Matsubara, Yuko

PATENT ASSIGNEE(S): Cellfree Sciences Co., Ltd., Japan

SOURCE: PCT Int. Appl., 27 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1 PATENT INFORMATION:

PATENT NO. KIND DATE APPLICATION NO. \_\_\_\_ \_\_\_\_\_ \_\_\_\_\_ WO 2006054683 A1 20060526 WO 2005-JP21216 20051118 W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD, MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM JP 2008035701 A 20080221 JP 2004-335392 20041119 JP 2004-335392 A 20041119 PRIORITY APPLN. INFO.:

ED Entered STN: 26 May 2006

AB It is intended to provide a system whereby an antibody in a specimen can be surely and quickly detected. To achieve this object, various attempts have been made to examine whether or not the cell-free protein synthesis method is applicable to this assay system. As an important factor for achieving the above object, various attempts have been made to improve the expression manner of a specific protein. As a result, it is found out that a target antibody in a specimen can be assayed by preparing a specific protein as a fused protein by the cell-free protein synthesis method and contacting this unpurified fused protein with the specimen.

REFERENCE COUNT: 15 THERE ARE 15 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 66 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2006:471739 CAPLUS <u>Full-text</u>

DOCUMENT NUMBER: 144:449469

TITLE: Cell-free protein synthesis process and apparatus INVENTOR(S): Endo, Yaeta; Sawasaki, Tatsuya; Ogasawara, Tomio;

Morishita, Ryo; Saeki, Mihoro

PATENT ASSIGNEE(S): Cellfree Sciences Co., Ltd., Japan

SOURCE: PCT Int. Appl., 35 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

	PATENT NO.				KIN	1D	DATE			APPLICATION NO.					DATE					
		2006	 50519	 301	=	 Z\ 1		2006	 50519	– R		 2005-	 TP2	 1715			 2005:	1111		
	NO											, BG,							CN,	,
CO,	CR,	CU,	CZ,	DE,	DK,	DM,	DZ,	EC,	EE,	EG,	ES,	FI,	GB,	GD,	GE,	GH,	GM,	HR,	HU,	ID,
IL,	IN,	IS,	JP,	KE,	KG,	KM,	KN,	KP,	KR,	KΖ,	LC,	LK,	LR,	LS,	LT,	LU,	LV,	LY,	MA,	MD,
			MG	, MK,	MN,	MW,	MX,	MZ,	, NA	, NG,	NI,	, NO,	NZ,	, OM,	PG,	PH	, PL	, PT	, RO	,
RU,	SC,	SD,	SE,	SG,	SK,	SL,	SM,	SY,	ТJ,	TM,	TN,	TR,	TT,	TZ,	UA,	UG,	US,	UZ,	VC,	VN,
YU,	ZA,	ZM,	ZW																	
		RW	: AT	, BE,	BG,	CH,	CY,	CZ,	, DE	, DK	EE,	, ES,	FI.	, FR,	GB,	GR	, HU	, IE	, IS	,
ΙΤ,	LT,	LU,	LV,	MC,	NL,	PL,	PT,	RO,	SE,	SI,	SK,	TR,	BF,	ΒJ,	CF,	CG,	CI,	CM,	GΑ,	GN,
GQ,	GW,	ML,	MR,	NE,	SN,	TD,	ΤG,	BW,	GH,	GM,	KE,	LS,	MW,	MZ,	NA,	SD,	SL,	SZ,	TZ,	UG,
			ZM	, ZW,	AM,	AZ,	BY,	KG,	, KZ	, MD,	, RU	, TJ,	, TM							
	JP	2008	30292	203		Α		2008	3021	4	JP :	2004-	-329	794			2004	1112		
PRI	ORIT	y api	PLN.	INF	).:						JP :	2004-	-329	794		Α .	2004	1112		

ED Entered STN: 21 May 2006

AB The cell-free protein synthesis comprises using directly the transcription products without the purification of the mRNA. The method is highly efficient and easy. Preparation of S-30 fraction of wheat germ of the cell-free translation was shown.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 67 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2006:469852 CAPLUS <u>Full-text</u>

DOCUMENT NUMBER: 144:447592

TITLE: Cell-free protein synthesizing bioreactor apparatus using

repetitive overlaying or repetitive batch-supplying system

INVENTOR(S): Endo, Yaeta; Sawasaki, Tatsuya; Ogasawara, Tomio

PATENT ASSIGNEE(S): Cellfree Sciences Co., Ltd., Japan

SOURCE: PCT Int. Appl., 44 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO. KIND DATE APPLICATION NO. DATE

WO 2006051908 A1 20060518 WO 2005-JP20727 20051111

W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,

CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID,

IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, LY, MA, MD,

MG, MK, MN, MW, MX, MZ, NA, NG, NI, NO, NZ, OM, PG, PH, PL, PT, RO,

RU, SC, SD, SE, SG, SK, SL, SM, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW

RW: AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IS, IT, LT, LU, LV, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG, BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM

JP 2008029204 A 20080214 JP 2004-329798 20041112 PRIORITY APPLN. INFO.: JP 2004-329798 A 20041112

ED Entered STN: 19 May 2006

A novel cell free protein synthesis system that has been improved for rapid AΒ and simple operation by omitting the use of complicated compartments of membrane filtration or column chromatogs. is provided. The bioreactor uses repetitive overlaying or repetitive batch-supplying system for operation. The system can monitor the reaction rate in its reaction chamber and the reaction rate is controlled by the supply rate (volume/s) of new reaction batch in a continuous or an discontinuous manner. A new reaction batch is designed to be added (overlayered) to (on) the reaction mixture and mixed in the chamber upon the detection of the reduced synthesis rate (at 10 min .apprx. 10 h time intervals). The reaction batch contain purified or coarse posttranscriptional mRNA fraction and wheat germ lysate (endosperm and inhibitor protein free and controlled sugar-phosphate levels). Condensation mechanism is used to remove reaction byproducts and excess reagents such as magnesium ion and nucleotides. Production of model protein (GFP) by the system (2 .apprx. 4 time repetitive reactions) was 1.1 .apprx. 1.8 mg/mL reaction mixture

REFERENCE COUNT: 14 THERE ARE 14 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 68 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2006:240332 CAPLUS Full-text DOCUMENT NUMBER: 145:119893

TITLE: Sequence-based analyses of biosynthesis rate limiting

factors in wheat germ cell-free system

Fujita, Naoya; Kinoshita, Kengo; Seki, Mutoaki; Sawasaki,

Tatsuya; Nakai, Kenta; Shinozaki, Kazuo; Endo, Yaeta

CORPORATE SOURCE: Human Genome Center, Institute of Medical Science, University of Tokyo, 4-6-1 Shirokanedai, Minato-ku, Tokyo, 108-8639, Japan

SOURCE: Genome Informatics 2005, International Conference, Poster

and Software Demonstrations, 16th, Yokohama, Japan, Dec. 19-21, 2005 (2005),

P095/1-P095/2. Universal Academy Press: Tokyo, Japan.

CODEN: 69HXB6

DOCUMENT TYPE: Conference LANGUAGE: English Entered STN: 17 Mar 2006 ED

AB The production of proteins is essential for their structural and functional analyses in the post-genome era. A wheat germ cell-free system is a useful method for this purpose. This system is able to produce proteins from various sources' mRNAs using the translational machinery from wheat germs. One improvement made in this system is the use of only one 5'-UTR, which is important in biosynthesis initiation, and one 3'-UTR for the expression of all coding sequences. However, depending on the coding sequences, the range of yields observed is very wide. The causes of the yield variation based on the protein sequences were investigated using a dataset consisting of 425 protein kinases from Arabidopsis thaliana. Based on the results obtained, two yield decreasing factors were identified: disorder and coiled coil.

THERE ARE 3 CITED REFERENCES AVAILABLE FOR THIS REFERENCE COUNT: 3 RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

ANSWER 69 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN L7ACCESSION NUMBER: 2006:117096 CAPLUS Full-text

DOCUMENT NUMBER: 144:186045

Nucleic acid sequences having translation enhancement TITLE:

activity and use

INVENTOR(S): Sawasaki, Tatsuya; Endo, Yaeta; Kamura, Nami

PATENT ASSIGNEE(S): Cellfree Sciences Co., Ltd., Japan

U.S. Pat. Appl. Publ., 16 pp. SOURCE:

CODEN: USXXCO

DOCUMENT TYPE: Patent LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.		DATE
				_	
US 20060029999	A1	20060209	US 2005-53594		20050208
JP 2006042676	A	20060216	JP 2004-227866		20040804
PRIORITY APPLN. INFO.:			JP 2004-227866	Α	20040804

ED Entered STN: 09 Feb 2006

AΒ The present invention provides a polynucleotide comprising a nucleic acid sequence having an activity of regulating the translation efficiency of a template in a cell-free protein synthesis system and also provides a method for utilizing the same, etc. Protein synthesis is carried out by a translation template containing a polynucleotide comprising a nucleic acid sequence which is to be an object to be selected, a polyribosome fraction is prepared from the reaction solution and a nucleic acid sequence bonding to ribosome is analyzed whereupon a selection is done.

ANSWER 70 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2005:1013930 CAPLUS Full-text

DOCUMENT NUMBER: 144:306950 TITLE: The wheat germ cell-free expression system: Methods for

high-throughput materialization of genetic information

AUTHOR(S): Sawasaki, Tatsuya; Gouda, Mudeppa D.; Kawasaki, Takayasu;

Tsuboi, Takafumi; Tozawa, Yuzuru; Takai, Kazuyuki; Endo, Yaeta

CORPORATE SOURCE: Cell-Free Science and Technology Research Center, Ehime

University, Matsuyama, Japan

SOURCE: Methods in Molecular Biology (Totowa, NJ, United States)

(2005), 310(Chemical Genomics), 131-144

CODEN: MMBIED; ISSN: 1064-3745

PUBLISHER: Humana Press Inc.

DOCUMENT TYPE: Journal LANGUAGE: English ED Entered STN: 20 Sep 2005

AB This chapter contains protocols for high-throughput protein production based

on the cell-free system prepared from eukaryote wheat embryos.

REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS

RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 71 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2005:612456 CAPLUS Full-text

DOCUMENT NUMBER: 143:128797

TITLE: Regulation of ATP mediated phosphorylation of carbohydrate

for increase the efficiency of cell-free protein synthesis INVENTOR(S): Endo, Yaeta; Ogasawara, Tomio

PATENT ASSIGNEE(S): Cellfree Sciences Co., Ltd., Japan

SOURCE: PCT Int. Appl., 39 pp.

CODEN: PIXXD2

KIND DATE

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

DATENT NO

	PAIENI NO.					VII	DAIE				APPLICATION NO.					DAIE				
	WO	200!	50639	979	_	A1	L	2005	5071	4	WO :	2004-	-JP1	 8928			2004	1217		
		W:	AE,	, AG,	AL,	AM,	AT,	, AU,	, AZ,	, BA	, BB	, BG,	, BR	, BW	, BY	, BZ	, CA	, CH	, CN	,
CO,	CR,	CU,	CZ,	DE,	DK,	DM,	DZ,	EC,	EE,	EG,	ES,	FI,	GB,	GD,	GE,	GH,	GM,	HR,	HU,	ID,
IL,	IN,	IS,	JP,	KE,	KG,	KP,	KR,	KΖ,	LC,	LK,	LR,	LS,	LT,	LU,	LV,	MA,	MD,	MG,	MK,	MN,
			MW.	, MX,	MZ,	NA,	NI,	NO,	, NZ,	, OM	, PG	, PH,	, PL	, PT	, RO	, RU	, SC	, SD	, SE	,
SG,	SK,	SL,	SY,	ΤJ,	TM,	TN,	TR,	TT,	TZ,	UA,	UG,	US,	UZ,	VC,	VN,	YU,	ZA,	ZM,	ZW	
		RW	: BW	, GH,	GM,	KE,	LS,	, MW	, MZ,	, NA	, SD	, SL,	, SZ	, TZ	, UG	, ZM	, ZW	, AM	, AZ	,
BY,	KG,	KΖ,	MD,	RU,	ΤJ,	TM,	ΑT,	BE,	BG,	CH,	CY,	CZ,	DE,	DK,	EE,	ES,	FΙ,	FR,	GB,	GR,
HU,	IE,	IS,	ΙΤ,	LT,	LU,	MC,	NL,	PL,	PT,	RO,	SE,	SI,	SK,	TR,	BF,	ВJ,	CF,	CG,	CI,	CM,
			GA,	, GN,	GQ,	GW,	ML,	, MR,	, NE,	, SN	, TD	, TG								
	US	200	70143	1661		A1	L	200	70623	1	US :	2006-	-596	538			2006	0710		
PRI	ORIT	Y API	PLN.	INFO	) <b>.:</b>						JP :	2003-	-434	080		Α .	2003	1226		
											WO :	2004-	-JP1	8928		W	2004	1217		

APPLICATION NO

חעדד

ED Entered STN: 15 Jul 2005

AB This invention provides a cell extract to increase the efficiency of cell-free protein synthesis by identifying and removing inhibitory and unstable contaminants in various existing cell exts.. The ATP-mediated carbohydrate phosphorylation system in the cell extract is regulated. The process consists of 1 removal of monosaccharides; 2 removal of phosphorylated saccharides; 3 regulation of the formation of monosaccharides from polysaccharides; and 4 regulation of the formation of phosphorylated saccharides from monosaccharides.

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

ACCESSION NUMBER: 2005:478393 CAPLUS Full-text

DOCUMENT NUMBER: 144:145607

TITLE: in vitro translation systems AUTHOR(S): Sawasaki, Tatsuya; Endo, Yaeta

CORPORATE SOURCE: Research Center of Cell-Free Bio-Science and Bio-

Engineering, Ehime University, Japan

Shokubutsu Saibo Kogaku Shirizu (2005), 21 (Moderu

Shokubutsu no Jikken Purotokoru (3rd Edition)), 230-234

CODEN: SSKSFR

PUBLISHER: Shujunsha

DOCUMENT TYPE: Journal; General Review

LANGUAGE: Japanese Entered STN: 06 Jun 2005 ED

A review. A typical protocol of the recombinant protein production in the AΒ cell free protein translation system using wheat germ extract was presented. The coverage of the procedures included the preparation of the wheat germ extract, the preparation of the template plasmid DNA (pEU: plasmid of Ehime University) for expressing recombinant fusion protein with GST, mRNA purifn., in vitro protein synthesis and the purification by using an affinity resin.

ANSWER 73 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN T.7 2005:300592 CAPLUS Full-text ACCESSION NUMBER:

DOCUMENT NUMBER: 142:353880

Producing antigens in wheat germ cell-free protein TITLE:

synthesis system for vaccine preparation

Endo, Yaeta; Tsuboi, Takafumi; Torii, Motomi; Sawasaki, INVENTOR(S):

Tatsuya

PATENT ASSIGNEE(S): Cellfree Sciences Co., Ltd., Japan

PCT Int. Appl., 39 pp. SOURCE:

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

P	ATENT NO.	KIND DAT	E A	APPLICATION NO.	DATE
M				70 2004-JP13918	
00 OD		·		BB, BG, BR, BW, BY,	
CO, CR	, CU, CZ, DE, DK,	DM, DZ, EC,	EE, EG, E	S, FI, GB, GD, GE,	GH, GM, HR, HU, ID,
IL, IN	, IS, JP, KE, KG,	KP, KR, KZ,	LC, LK, L	R, LS, LT, LU, LV,	MA, MD, MG, MK, MN,
	MW, MX, MZ,	NA, NI, NO	NZ, OM,	PG, PH, PL, PT, RO,	RU, SC, SD, SE,
SG, SK	, SL, SY, TJ, TM,	TN, TR, TT,	TZ, UA, U	IG, US, UZ, VC, VN,	YU, ZA, ZM, ZW
	RW: BW, GH, GM,	KE, LS, MW	, MZ, NA,	SD, SL, SZ, TZ, UG,	ZM, ZW, AM, AZ,
BY, KG	, KZ, MD, RU, TJ,	TM, AT, BE,	BG, CH, C	CY, CZ, DE, DK, EE,	ES, FI, FR, GB, GR,
HU, IE	, IT, LU, MC, NL,	PL, PT, RO,	SE, SI, S	SK, TR, BF, BJ, CF,	CG, CI, CM, GA, GN,
	GQ, GW, ML,	MR, NE, SN	, TD, TG		
E	P 1669448	A1 200	60614 E	IP 2004-788077	20040924
	R: AT, BE, CH,	DE, DK, ES	FR, GB,	GR, IT, LI, LU, NL,	SE, MC, PT, IE,
SI, FI	, RO, CY, TR, BG,	CZ, EE, HU,	PL, SK		
U	S 20060233789	A1 200	61019 U	JS 2006-572139	20060411
PRIORI'	TY APPLN. INFO.:		J	TP 2003-333659	A 20030925
			W	7O 2004-JP13918	W 20040924

ED Entered STN: 07 Apr 2005

AΒ Provided here is a means of producing antigens retaining its native antigenicity by using a cell-free protein synthesis means. In particular, it provides a means of producing antigens without being ruled by codon usage via, for example, from an AT-rich gene. Antibodies produced with the vaccine, and use as diagnostic agent, and kit are also disclosed. Screening of

antimalarials is also disclosed. Antigens retaining its antigenicity (in particular, a malaria antigens useful in producing malaria vaccines) were successfully prepd. with wheat germ cell-free protein synthesis system. Transmission-blocking vaccines target the sexual stages of the malaria parasite and prevent further development within the mosquito vector halting the transmission of the parasite. Zygote/ookinetes are potential targets of antibodies inhibiting oocyst development in the mosquito midgut and rendering mosquitoes non-infectious. DNA vaccine constructs were developed expressing Pvs25 and Pvs28 (Plasmodium vivax zygote/ookinete surface proteins). Antibodies produced in mice after immunization recognized resp. antigens, and these antibodies when tested in assay were potent blockers of P. vivax transmission.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 74 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2005:297489 CAPLUS <u>Full-text</u>

DOCUMENT NUMBER: 142:332432

TITLE: Preparation of cell extracts for cell-free translation INVENTOR(S): Tozawa, Yuzuru; Kanno, Takuya; Endo, Yaeta; Dohi, Naoki;

Koga, Hirohisa

PATENT ASSIGNEE(S): Zoegene Corporation, Japan SOURCE: Jpn. Kokai Tokkyo Koho, 14 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2005087208	A	20050407	JP 2004-228349	20040804
PRIORITY APPLN. INFO.:			JP 2003-289874 A	20030808

ED Entered STN: 07 Apr 2005

AB Cells are segmented, and mixed with extraction solvents to prepare cell exts. In the extraction process, an activation agents selected from aluminum oxide, activated aluminum oxide, silica gel, etc., is added for activation of the cell exts. The cells are preferably plant germ cells, especially wheat germ cells. The segmentation is achieved by impact or cutting. The method provides enhanced cell-free translation with high efficiency. Preparation of wheat germ extract by Waring blendor, activation of the wheat germ extract with aluminum oxide, and enhanced cell-free translation of green fluorescence protein (GFP) were shown.

L7 ANSWER 75 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2005:239233 CAPLUS <u>Full-text</u>

DOCUMENT NUMBER: 142:291317

TITLE: Novel high throughput screening method of drug for

physiologically active protein

INVENTOR(S): Endo, Yaeta; Sawasaki, Tatsuya
PATENT ASSIGNEE(S): Cellfree Sciences Co., Ltd., Japan

SOURCE: PCT Int. Appl., 47 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO. KIND DATE APPLICATION NO. DATE

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WO 2005024428
                        A1
                               20050317
                                          WO 2004-JP13071
                                                                  20040908
        W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN,
CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID,
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            MW, MX, MZ, NA, NI, NO, NZ, OM, PG, PH, PL, PT, RO, RU, SC, SD, SE,
SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
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HU, IE, IT, LU, MC, NL, PL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN,
            GQ, GW, ML, MR, NE, SN, TD, TG
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                                          EP 2004-787754
    EP 1669759
                         Α1
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SI, FI, RO, CY, TR, BG, CZ, EE, HU, PL, SK
                               20060810
                                           US 2006-571081
    US 20060177813
                       A1
                                                                  20060331
PRIORITY APPLN. INFO.:
                                           JP 2003-316081
                                                               A 20030908
                                           WO 2004-JP13071
                                                               W 20040908
    Entered STN: 18 Mar 2005
ED
AΒ
     A safe and rapid means for screening a drug (in particular, an inhibitor) for
     a physiol. active protein is provided, with which a system for synthesizing a
     physiol. active protein sustaining its activity is constructed by utilizing a
     wheat germ extract cell-free protein synthesis system among cell-free protein
     synthesis means. As a typical example of using this synthesis system, a
     system for screening a SARS 3CLpro inhibitor is constructed.
                       7
                              THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS
REFERENCE COUNT:
RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT
    ANSWER 76 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN
L7
ACCESSION NUMBER:
                        2004:965399 CAPLUS Full-text
DOCUMENT NUMBER:
                        141:391506
TITLE:
                        Automatic process and apparatus for high throughput cell-
free protein synthesis utilizing membrane filter concentration
                       Endo, Yaeta; Sawasaki, Tatsuya; Ogasawara, Tomio;
INVENTOR(S):
Morishita, Riyo; Saeki, Mihoro; Sato, Tomohisa; Kitamoto, Aya
PATENT ASSIGNEE(S):
                        Japan
SOURCE:
                        PCT Int. Appl., 81 pp.
                        CODEN: PIXXD2
DOCUMENT TYPE:
                        Patent
LANGUAGE:
                        Japanese
FAMILY ACC. NUM. COUNT:
PATENT INFORMATION:
    PATENT NO.
                       KIND DATE
                                      APPLICATION NO.
                               _____
                                          _____
    WO 2004097014
                        A1
                               20041111
                                          WO 2004-JP5912
                                                                  20040423
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SG, SK, SL, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, YU, ZA, ZM, ZW
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    JP 2006042601
                         Α
                               20060216
                                           JP 2003-281500
                                                                  20030729
    AU 2004234669
                               20041111
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                                                                  20040423
    CA 2522927
                         Α1
                               20041111
                                           CA 2004-2522927
                                                                  20040423
    EP 1619247
                         Α1
                               20060125
                                           EP 2004-729258
                                                                  20040423
        R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE,
SI, LT, LV, FI, RO, MK, CY, AL, TR, BG, CZ, EE, HU, PL, SK, HR
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CN 1777674	A	20060524	CN	2004-80010994		20040423
US 20060257997	A1	20061116	US	2005-554434		20051214
PRIORITY APPLN. INFO.:			JP	2003-122930	Α	20030425
			JP	2003-281500	Α	20030729
			WO	2004-JP5912	W	20040423

ED Entered STN: 12 Nov 2004

AΒ A method and automated apparatus for high throughput in vitro synthesis of biopolymers such as proteins or RNA, are disclosed. The cell-free synthesis system comprises the following means: (1) a template material, a substrate and a reaction solution are contacted together and introduced into a synthesis reaction system. (2) before or after almost lowering the synthesis speed, or almost ceasing the synthesis reaction, or during the same, the reaction system is taken out from the synthesis reaction system and the solution is diluted (3) following the dilution, it is concd. (4) the reaction system is returned into the synthesis reaction system. An alternative means is as follows. (1) a template material, a substrate and a reaction solution are contacted together and introduced into a synthesis reaction system. (2) before or after almost lowering the synthesis speed, or almost ceasing the synthesis reaction, or during the same, the reaction system is taken out from the synthesis reaction system and the solution is concentrated (3) following the concn., it is diluted (4) the diluted reaction system is returned into the synthesis reaction system. Concentration is accomplished with a membrane filter, centrifuge, and/or a suction pump. Information processing program for the automated apparatus is claimed.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 77 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2004:678409 CAPLUS Full-text

DOCUMENT NUMBER: 141:170409

TITLE: Automatic process for protein synthesis and apparatus

therefor

INVENTOR(S): Endo, Yaeta; Sawasaki, Tatsuya

PATENT ASSIGNEE(S): Japan

SOURCE: PCT Int. Appl., 41 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.						1D	DATE			APP1	LICAT	CION	NO.		I	DATE			
				_					-										
WO	2004	40700	047		A1	-			WO 2	2004-	-JP13	364		2	20040	0210			
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CR,	CU,	CZ,	DE,	DK,	DM,	DZ,	EC,	EE,	EG,	ES,	FΙ,	GB,	GD,	GE,	GH,	GM,	HR,	HU,	ID,
IN,	IS,	JP,	KE,	KG,	KP,	KR,	KΖ,	LC,	LK,	LR,	LS,	LT,	LU,	LV,	MA,	MD,	MG,	MK,	MN,
MW, MX, MZ						NΙ													
	WO	WO 2004 W: CR, CU,	WO 20040700 W: AE, CR, CU, CZ, IN, IS, JP,	WO 2004070047 W: AE, AG, CR, CU, CZ, DE, IN, IS, JP, KE,	WO 2004070047 W: AE, AG, AL, CR, CU, CZ, DE, DK, IN, IS, JP, KE, KG,	WO 2004070047 A1 W: AE, AG, AL, AM, CR, CU, CZ, DE, DK, DM, IN, IS, JP, KE, KG, KP,	WO 2004070047 A1 W: AE, AG, AL, AM, AT, CR, CU, CZ, DE, DK, DM, DZ,	WO 2004070047 A1 2004 W: AE, AG, AL, AM, AT, AU, CR, CU, CZ, DE, DK, DM, DZ, EC, IN, IS, JP, KE, KG, KP, KR, KZ,	WO 2004070047 A1 20040819 W: AE, AG, AL, AM, AT, AU, AZ, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, IN, IS, JP, KE, KG, KP, KR, KZ, LC,	WO 2004070047 A1 20040819 W: AE, AG, AL, AM, AT, AU, AZ, BA CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK,	WO 2004070047 A1 20040819 WO : W: AE, AG, AL, AM, AT, AU, AZ, BA, BB CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR,	WO 2004070047 A1 20040819 WO 2004- W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS,	WO 2004070047 A1 20040819 WO 2004-JP13 W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT,	WO 2004070047 A1 20040819 WO 2004-JP1364 W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU,	WO 2004070047 A1 20040819 WO 2004-JP1364 W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV,	WO 2004070047 A1 20040819 WO 2004-JP1364  W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ  CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH,  IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA,	WO 2004070047 A1 20040819 WO 2004-JP1364 20040 W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD,	WO 2004070047 A1 20040819 WO 2004-JP1364 20040210 W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG,	WO 2004070047 A1 20040819 WO 2004-JP1364 20040210 W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BW, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, HR, HU, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK,

RW: BW, GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZM, ZW, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HU, IE, IT, LU, MC, NL, PT, RO, SE, SI, SK, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG
PRIORITY APPLN. INFO.:

JP 2003-33009

A 20030210

ED Entered STN: 19 Aug 2004

AB In cell-free protein synthesis, the transcription template is precipitated and dried while the supernatant been removed. The transcription template is easily solubilized in the cell -free protein synthesis system. The low-mol. weight protein synthesis inhibitors in the plant seed germ extract such as wheat germ extract and albumen is removed prior to the cell-free protein synthesis. The method is highly efficient; the yield of mRNA is high; and loss of mRNA is minimal. An automated cell-free protein synthesis apparatus

comprising temperature control system, sample supply system, precipitation means, supernatant removal system, drying system. etc., is also given.

ANSWER 78 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2004:80730 CAPLUS Full-text

DOCUMENT NUMBER: 140:144709

TITLE: Single chain antibody comprising light and heavy chains

coupled by a labeled linker for antigen immunoassay chip and test kit

Endo, Yaeta; Kawasaki, Takayasu; Sawasaki, Tatsuva INVENTOR(S):

PATENT ASSIGNEE(S): Japan

PCT Int. Appl., 63 pp. SOURCE:

CODEN: PIXXD2

Patent DOCUMENT TYPE: Japanese LANGUAGE:

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

	PATENT NO.				KII	dN	DATE			APPLICATION NO.				DATE						
	WO	200	4009	 639	_	A	1				WO	2003-	-JP9:	L40						
		W:	ΑE	, AG	, AL,	, AM	, AT,	AU,	, AZ,	, BA	, BB	, BG,	BR,	BY,	BZ,	, CA,	CH,	CN,	CO	,
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IS,	JP,	ΚE,	KG,	ΚP,	KR,	KΖ,	LC,	LK,	LR,	LS,	LT,	LU,	LV,	MA,	MD,	MG,	MK,	MN,	MW,	MX,
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SY,	ΤJ,	TM,	TN,	TR,	TT,	TZ,	UA,	UG,	US,	UZ,	VC,	VN,	YU,	ZA,	ZM,	ZW				
		RW	: GH	, GM	, KE,	LS	, MW,	MZ,	, SD,	, SL	, SZ	, TZ,	UG,	ZM,	ZW,	, AM,	AZ,	BY,	KG	,
KΖ,	MD,	RU,	ΤJ,	TM,	ΑT,	BE,	BG,	CH,	CY,	CZ,	DE,	DK,	EE,	ES,	FΙ,	FR,	GB,	GR,	HU,	ΙE,
ΙT,	LU,	MC,	NL,	PT,	RO,	SE,	SI,	SK,	TR,	BF,	ВJ,	CF,	CG,	CI,	CM,	GΑ,	GN,	GQ,	GW,	ML,
			MR	, NE	, SN,	TD	, TG													
	CA	249.	2996			A.	1	2004	40129	9	CA	2003-	-2492	2996		2	20030	718		
	AU	200	3248	081		A.	1	2004	40209	9	AU	2003-	-2480	081		4	20030	718		
	EP	154	1588			A.	1	2005	5061	5	EP	2003-	-7653	324		4	20030	718		
		R:	ΑT	, BE	, CH,	DE	, DK,	ES,	FR,	, GB	, GR	, IT,	LI,	LU,	NL,	, SE,	MC,	PT,	IE,	,
SI,	LT,	LV,	FI,	RO,	MK,	CY,	AL,	TR,	BG,	CZ,	EE,	HU,	SK							
	US	200	6017.	2344		A.	1	2006	50803	3	US	2005-	-5220	000		4	20050	)223		
PRI	ORIT	Y AP	PLN.	INF	0.:						JP	2002-	-2100	067		A 2	20020	718		
												2003-	-JP91	L40		W 2	20030	718		

Entered STN: 01 Feb 2004 ED

It is intended to provide a single chain antibody sustaining an activity of AΒ specifically binding to an antigen and a labeled single chain antibody composed of the single chain antibody and a label bonded thereto. More specifically, the labeled single chain antibody as described above can be produced by bonding a label to the linker moiety of the single chain antibody. This antibody is produced by using a wheat germ-origin cell free protein synthesis system in a less reductive state where a disulfide bond in a mol. can be maintained. Further, the antibody is bonded to a solid phase via the label to thereby produce an immobilized single chain antibody. Also, an antigen-antibody reaction is analyzed with the use of this immobilized single chain antibody.

REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

ANSWER 79 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2003:938880 CAPLUS Full-text

DOCUMENT NUMBER: 139:394969

Selection of germs from plant seeds, manufacture of the germ extracts, and method, solutions, and kits for cell-free protein synthesis using the extracts

INVENTOR(S): Dohi, Naoki; Morisada, Shigeo; Iwahashi, Shigeo; Endo, Yaeta

PATENT ASSIGNEE(S): Japan

SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2003339395 PRIORITY APPLN. INFO.:	A	20031202	JP 2002-152245 JP 2002-152245	20020527 20020527

ED Entered STN: 02 Dec 2003

Intact germs, from which exts. are prepared for cell-free protein synthesis, AΒ are selected by milling plant seeds using an impact mill at impact velocity 25-70 m/s and separating germs from the milled product. Germ exts. are manufactured by pulverizing the above selected germs and extracting the germs. Also claimed are a cell-free protein synthesis method using the germ exts., solns. containing the germ exts.,  $\mbox{ATP}$  ,  $\mbox{GTP}$ , creatine phosphate, creatine kinase, L-amino acids, K+, and Mg2+, and reagent kits containing the germ exts. or the solns. Wheat was hammer-milled at impact velocity 35 m/s and sieved to recover a 0.7-1.1-mm germ-containing fraction. The fraction was fed to a pneumatic classifier to remove bran, endoderm, etc. Germs having germinating capacity were separated from the fraction by heavy medium separation using CC14/cyclohexane (2.4:1), dried, and remaining brans were removed by winnowing. The crude germ fraction was further separated into germs and seed coat/endosperm by a method based on difference in color. The separated germs were ultrasonically cleaned in  ${\tt H2O}$  at  ${\tt 4^{\circ}}$  and further ultrasonically cleaned in Nonidet solution to completely remove endosperm component. The cleaned germs were extracted with a soln. containing HEPES-KOH, AcOK, Mg(OAc)2, CaCl2, L-amino acids, and dithiothreitol in a Waring blender. The mixture was centrifuged and the supernatant was gel-filtered, and the filtrate was centrifuged again to give wheat germ extract Synthesis of green fluorescent protein using the extract was also shown.

L7 ANSWER 80 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2003:936309 CAPLUS <u>Full-text</u>

DOCUMENT NUMBER: 139:394968

TITLE: Manufacture of germ extracts, solution containing them,

and method and kit for cell-free protein synthesis using the solution

INVENTOR(S): Dohi, Naoki; Morisada, Shigeo; Iwahashi, Shigeo; Endo.

Yaeta

PATENT ASSIGNEE(S): Japan

SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2003339394	A	20031202	JP 2002-152236	20020527
PRIORITY APPLN. INFO.:			JP 2002-152236	20020527

ED Entered STN: 02 Dec 2003

AB In manufacture of germ exts. by milling plant seeds, separating germs, washing the germs, grinding the germs, and extracting the germs, the germs are contacted with nonchlorine solvents prior to grinding to remove lipids. Also

claimed are cell- free protein synthesis solns. containing the germ exts., method to cell-free protein synthesis using the solns., and cell-free protein synthesis kits containing the solns. Wheat germs were recovered from milled wheat by sieving and winnowing and fed to an electromagnetic feeder having an vibrator to sep. crude germ fraction. The crude fraction was further separated into germs and seed coat/endosperm by a method based on difference in color. The separated germs were treated with EtOH for 5 min, dried, ultrasonically cleaned in H2O at  $4^{\circ}$ , and further ultrasonically cleaned in Nonidet solution The cleaned germs were extracted with a solution containing HEPES-KOH, AcOK, Mg(OAc)2, CaCl2, L-amino acids, and dithiothreitol in a Waring blender. The mixture was centrifuged and the supernatant was gelfiltered, and the filtrate was centrifuged again to give wheat germ extract Synthesis of green fluorescent protein using the extract was also shown.

ANSWER 81 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2003:913316 CAPLUS Full-text

DOCUMENT NUMBER: 139:360695

TITLE: Lyophilized preparation for cell-free protein synthesis

INVENTOR(S): Endo, Yaeta; Ogasawara, Tomio

Japan PATENT ASSIGNEE(S):

SOURCE: PCT Int. Appl., 32 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent Japanese LANGUAGE:

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

	PA.	TENT	NO.			KII	MD.		E -		APP	LICAT	CION	NO.			DATE			
	WO	200	3095	 661	_	A.	1				WO	2003-	-JP56	556						
		W:	ΑE	, AG	, AL,	, AM	, AT,	AU,	, AZ	, BA	, BB	, BG,	BR,	BY,	BZ,	CA,	CH,	, CN,	CO	,
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IS,	JP,	ΚE,	KG,	KP,	KR,	KΖ,	LC,	LK,	LR,	LS,	LT,	LU,	LV,	MA,	MD,	MG,	MK,	MN,	MW,	MX,
			MZ	, NI	NO,	NZ,	, OM,	PH,	PL,	, PT	, RO	, RU,	SC,	SD,	SE,	SG,	SK,	, SL,	TJ	,
TM,	TN,	TR,	TT,	TZ,	UA,	UG,	US,	UZ,	VC,	VN,	YU,	ZA,	ZM,	ZW						
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KΖ,	MD,	RU,	ТJ,	TM,	ΑT,	BE,	BG,	CH,	CY,	CZ,	DE,	DK,	EE,	ES,	FI,	FR,	GB,	GR,	HU,	ΙE,
ΙΤ,	LU,	MC,	NL,	PT,	RO,	SE,	SI,	SK,	TR,	BF,	ВJ,	CF,	CG,	CI,	CM,	GΑ,	GN,	GQ,	GW,	ML,
			MR	, NE	SN,	. TD	, TG													
	AU	200	3235	844		A.	1	2003	3111:	1	AU	2003-	-2358	344		2	20030	0506		
	CA	248	5827			A.	1	2003	31120	C	CA	2003-	-2485	5827		2	20030	0506		
	EP	155	0728			A.	1	2005	50706	6	EP	2003-	-721(	030		2	20030	0506		
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SI,	LT,	LV,	FΙ,	RO,	MK,	CY,	AL,	TR,	BG,	CZ,	EE,	HU,	SK							
	US	200	5015.	3390		A.	1	2005	5071	4	US	2004-	-5148	355		2	20041	1221		
PRI	ORIT	Y AP	PLN.	INF	0.:						JΡ	2002-	-1388	328		A 2	20020	0514		
											WO	2003-	-JP56	556		W 2	20030	0506		

ED Entered STN: 21 Nov 2003

AΒ The lyophilized cell-free protein synthesis system has comparable activity to that prepared by low-temperature preservation. The cell-free protein synthesis system contains lower deliquescent substances such as potassium acetate. The deliquescent substances amount to  $\leq 0.01$  weight% based on 1 weight% protein in the cell-free protein synthesis system. The low-mol. weight protein synthesis inhibitors are removed in the presence of high-energy phosphate compds. such as ATP.

THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS REFERENCE COUNT: 9 RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

ACCESSION NUMBER: 2003:832735 CAPLUS Full-text

DOCUMENT NUMBER: 141:19197

TITLE: High-throughput functional and structural analysis of

proteins based on cell-free protein synthetic technology AUTHOR(S): Endo, Yaeta; Sawasaki, Tatsuya

CORPORATE SOURCE: Faculty of Engineering, Department of Applied Chemistry,

Ehime University, Matsuyama, 790-8577, Japan

SOURCE: Biobencha (2003), 3(5), 22-24 CODEN: BIOBC8; ISSN: 1346-5376

PUBLISHER: Yodosha

DOCUMENT TYPE: Journal; General Review

LANGUAGE: Japanese ED Entered STN: 24 Oct 2003

AB A review. Advantages of the use of cell-free protein translation system to prepare stable protein samples efficiently for structural analyses were discussed. Tech. break through to improve production efficiency of wheat germ lysate cell-free system was described. Application of the technol. to high-throughput screening for functional and structural anal. of proteins was discussed. Development of automatized protein synthesis robot system was also discussed.

L7 ANSWER 83 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2003:697078 CAPLUS Full-text

DOCUMENT NUMBER: 139:210387

TITLE: Reaction solution for and preparation of cell-free protein

synthesis system for manufacture of protein and antibody

KIND

INVENTOR(S): Endo, Yaeta; Kawasaki, Takayasu; Sawasaki, Tatsuya

PATENT ASSIGNEE(S): Japan

SOURCE: PCT Int. Appl., 62 pp.

CODEN: PIXXD2

בות עכו

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

OM TEMPT NO

						KIL	עו	DAIL	5			LICA.				_	JAIL			
						 A1	 L	2003	30904	4		2003-					2003	0228		
		W:	ΑE	, AG,	, AL,	AM,	AT,	AU,	AZ,	, BA,	, BB	, BG,	BR	, BY,	, BZ,	, CA,	CH,	, CN,	CO	,
CR,	CU,	CZ,	DE,	DK,	DM,	DZ,	EC,	EE,	ES,	FI,	GB,	GD,	GE,	GH,	GM,	HR,	HU,	ID,	IL,	IN,
IS,	JP,	ΚE,	KG,	KR,	KΖ,	LC,	LK,	LR,	LS,	LT,	LU,	LV,	MA,	MD,	MG,	MK,	MN,	MW,	MX,	MZ,
			NO	, NZ,	, OM,	PH,	PL,	PT,	RO,	, RU,	, SC	, SD,	SE,	, SG,	, SK,	, SL,	. TJ	, TM,	TN	,
TR,	TT,	TZ,	UA,	UG,	US,	UZ,	VC,	VN,	YU,	ZA,	ZM,	ZW								
		RW	: GH	, GM,	, KE,	LS,	MW,	MZ,	SD,	, SL,	, SZ	, TZ,	. UG	, ZM,	, ZW,	, AM,	. AZ	, ву,	KG	,
KΖ,	MD,	RU,	ΤJ,	TM,	ΑT,	BE,	BG,	CH,	CY,	CZ,	DE,	DK,	EE,	ES,	FI,	FR,	GB,	GR,	HU,	ΙE,
ΙT,	LU,	MC,	NL,	PT,	SE,	SI,	SK,	TR,	BF,	ВJ,	CF,	CG,	CI,	CM,	GΑ,	GN,	GQ,	GW,	ML,	MR,
			NE	, SN,	, TD,	TG														
	CA	247	7440			A1	L	2003	30904	4	CA	2003-	-247	7440		4	2003	0228		
	AU	200	3211	458		A1	L	2003	30909	9	AU	2003-	-211	458		2	2003	0228		
	EP	148	9188			A1	L	2004	11222	2	EP	2003-	-7430	063		4	2003	0228		
		R:	AT	, BE,	CH,	DE,	DK,	ES,	FR,	, GB,	, GR	, IT,	LI	, LU,	, NL,	, SE,	MC.	, PT,	IE	,
SI,	LT,	LV,	FI,	RO,	MK,	CY,	AL,	TR,	BG,	CZ,	EE,	HU,	SK							
	US	200	5014	8046		A1	L	2005	5070	7	US	2004-	-506	127		4	2004	1104		
	US	727.	3615			B2	2	200	7092	5										
PRI	ORIT	Y AP	PLN.	INFO	) <b>.:</b>						JP	2002-	-531	61		A 2	2002	0228		
											WO	2003-	-JP2	313		W 2	2003	0228		

ADDITCATION NO

חת עם

ED Entered STN: 05 Sep 2003

AB The cell-free protein synthesis system (redox potential, -100 to 0 mV) is useful for formation of intramol. disulfide linkage and folding of proteins,

especially antibodies. The cell-free protein synthesis system contains  $\geq 1$  reductants selected from dithiothreitol, 2-mercaptoethanol, and glutathione. It further contains catalysts such as disulfide isomerase for disulfide exchange and formation of proper disulfide linkage. Manufacture of single-stranded antibody to Salmonella O-antigen with a wheat germ cell-free protein synthesis system was shown.

REFERENCE COUNT: 15 THERE ARE 15 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 84 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2003:610649 CAPLUS  $\underline{\text{Full-text}}$ 

DOCUMENT NUMBER: 139:145563

TITLE: "Ready-made" wheat germ cell-free protein synthesis system

INVENTOR(S):
Endo, Yaeta

PATENT ASSIGNEE(S): Japan

SOURCE: PCT Int. Appl., 55 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

	PATENT N	10.		KIND	DATE		APPLICA	TION NO.		DATE	
					20030807	7					
				•					•	, CH, CN,	•
CR, C	CU, CZ, I	DE, DK,	DM,	DZ, EC,	EE, ES,	FI,	GB, GD,	GE, GH,	GM, HR,	HU, ID,	IL, IN,
IS, J	TP, KE, F	KG, KR,	KΖ,	LC, LK,	LR, LS,	LT,	LU, LV,	MA, MD,	MG, MK,	MN, MW, I	MX, MZ,
		NO, NZ,	, OM,	PH, PL	, PT, RO,	RU	, SC, SD	, SE, SG,	SK, SL	, TJ, TM,	TN,
TR, T	II, TZ, t	JA, UG,	US,	UZ, VC,	VN, YU,	ZA,	ZM, ZW				
	RW:	GH, GM,	KE,	LS, MW	, MZ, SD,	SL	, SZ, TZ	, UG, ZM,	ZW, AM	, AZ, BY,	KG,
KZ, M	ID, RU, I	IJ, TM,	AT,	BE, BG,	CH, CY,	CZ,	DE, DK,	EE, ES,	FI, FR,	GB, GR,	HU, IE,
IT, L	JU, MC, N	NL, PT,	SE,	SI, SK,	TR, BF,	ВJ,	CF, CG,	CI, CM,	GA, GN,	GQ, GW, 1	ML, MR,
		NE, SN,	, TD,	TG							
	CA 24833	332		A1	20030807	7	CA 2003	-2483332		20030131	
	EP 1479	776		A1	20041124	Į	EP 2003	-734897		20030131	
										, MC, PT,	IE,
SI, L	T, LV, E	ri, RO,	MK,	CY, AL,	TR, BG,	CZ,	EE, HU,	SK	·		•
•			•		20050727					20030131	
	US 20050	064592		A1	20050324	Į.	US 2004	-503259		20041115	
	RITY APPI							-23141		20020131	
								-JP975			

ED Entered STN: 08 Aug 2003

AB The low-mol.-weight inhibitors (mol.-weight, ≤ 14000 dalton) to protein synthesis in the cell-free protein synthesis germ extract, especially wheat germ extract, is removed by dialysis using regenerated cellulose membrane or by gel filtration. The dialysis was done in the presence of high-energy phosphates such as ATP and/or amino acids for stabilization of the cell-free protein synthesis system. Removal of the low-mol.-weight inhibitors from wheat germ extract cell-free protein synthesis system with Spectrapor 6 (Spectrum Medical Ind., Inc.) was shown.

REFERENCE COUNT: 18 THERE ARE 18 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 85 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2003:610648 CAPLUS Full-text DOCUMENT NUMBER: 139:145562

TITLE: Germ extract for cell-free protein synthesis and process

for producing the same

INVENTOR(S): Endo, Yaeta; Dohi, Naoki; Nakagawa, Makoto

PATENT ASSIGNEE(S): Japan

SOURCE: PCT Int. Appl., 45 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

	PA'	TENT	NO.			KIN	1D	DATE	C		APP:	LICAT	NOI	NO.		Γ	DATE			
	WO											 2003- , BG,					2003(		. (0	
		CZ,	DE,	DK,	DM,	DZ,	EC,	EE,	ES,	FI,	GB,	GD, LV,	GE,	GH,	GM,	HR,	HU,	ID,	IL,	IN,
ŕ	ŕ	·	NO.	, NZ,	OM,	PH,	PL,	PT,	RO,	, RU,	, sc	, SD,	•		•	•		•	,	•
		RW		, GM,	KE,	LS,	MW,	MZ,	SD,	, SL,	, SZ	, TZ,								
				•								DK, CG,								•
	CA	247	NE, 4466	, SN,	,		L	2003	3080	7	CA .	2003-	-2474	1466		2	20030	0131		
			7566 7566								EP .	2003-	-7349	00		2	20030	)131		
ST.	T.T.				,			•	,			, IT, HU,		LU,	NL,	SE,	MC,	PT,	ΙE	,
51,	JP	•	6780	·	,	В2	2	2006	0215	5	JP .	2003- 2003-	-5642							
	US	200	50042	2305		A1	L	2005	0224	4	US .	2004-	-5032	271		2	20041	1025		
			5211 6314			A B2		2005			JP .	2005-	-6294	łΙ		2	20050	)307		
PRI	ORIT	Y AP	PLN.	INF	).:							2002- 2002-								
												2002- 2003-								
												2003-					20030			

ED Entered STN: 08 Aug 2003

AB Germ exts. of wheat, barley, rice, and corn are prepared by impacting, and/or cutting in the presence/absence of extraction solvent for stable and high-efficiency cell-free protein synthesis system. The germ exts. have reduced RNase activity ( $\leq 10$  pg/ $\mu$ l). The DNA and total fatty acids in the germ exts. are 230  $\mu$ g/mL and  $\leq$  0.03 g/100g, resp., when the (optical d., 0. D.) A260 = 90. It does not require the addition of t-RNA, and is low in impurities such as organelles, cell wall, etc. Manufacture of dihydrofolic acid dehydrogenase using the wheat germ cell-free protein synthesis system was shown.

REFERENCE COUNT: 23 THERE ARE 23 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 86 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2003:532764 CAPLUS Full-text

DOCUMENT NUMBER: 139:80165

TITLE: Isolating nucleotide sequence regulating the translation

efficiency in cell-free protein synthesis system

INVENTOR(S): Endo, Yaeta; Sawasaki, Tatsuya

PATENT ASSIGNEE(S): Japan

SOURCE: PCT Int. Appl., 89 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

	PAT	rent	NO.			KIN	1D		<u> </u>			LICAT					DATE			
	WO	2003	 30560	 009	_	 A1	 L					2002-					2002:	1227		
		W:	ΑE,	, AG,	, AL,	, AM,	AT,	AU,	, AZ	, BA,	, BB	, BG,	, BR,	BY,	BZ,	CA,	, CH	, CN,	, CO,	,
CR,	CU,	CZ,	DE,	DK,	DM,	DZ,	EC,	EE,	ES,	FI,	GB,	GD,	GE,	GH,	GM,	HR,	HU,	ID,	IL,	IN,
IS,	JP,	ΚE,	KG,	KR,	KΖ,	LC,	LK,	LR,	LS,	LT,	LU,	LV,	MA,	MD,	MG,	MK,	MN,	MW,	MX,	MΖ,
			NO,	, NZ,	, OM,	, PH,	PL,	PT,	RO,	, RU,	, SC	, SD,	, SE,	SG,	SK,	SL,	, TJ	, TM,	, TN,	,
TR,	TT,	TZ,	UA,	UG,	US,	UΖ,	VC,	VN,	YU,	ZA,	ZM,	ZW								
		RW	: GH,	, GM,	, KE,	, LS,	MW,	MZ,	SD,	, SL,	, SZ	, TZ,	, UG,	ZM,	ZW,	AM,	, AZ	, BY,	, KG	,
KΖ,	MD,	RU,	ΤJ,	TM,	ΑT,	BE,	BG,	CH,	CY,	CZ,	DE,	DK,	EE,	ES,	FI,	FR,	GB,	GR,	ΙE,	ΙT,
LU,	MC,	NL,	PT,	SE,	SI,	SK,	TR,	BF,	ВJ,	CF,	CG,	CI,	CM,	GΑ,	GN,	GQ,	GW,	ML,	MR,	NE,
			SN,	, TD,	, TG															
	CA	247	1667			A1	L	2003	3071	C	CA	2002-	-2471	1667		2	2002	1227		
	AU	2002	23671	144		A1	L	2003	3071	5	AU	2002-	-3671	l 4 4		2	2002	1227		
	EP	146	6972			A1	L	2004	11013	3	EP	2002-	-7909	919		2	2002	1227		
	EP	1466	6972			В1	L	200	70228	3										
		R:	AT,	, BE,	, СН,	, DE,	DK,	ES,	FR	, GB,	, GR	, IT,	, LI,	LU,	NL,	SE,	, MC	, PT,	, IE,	,
SI,	LT,	LV,	FI,	RO,	MK,	CY,	AL,	TR,	BG,	CZ,	EE,	SK								
	JP	3703	1292			В2	2	2005	50928	8	JP	2003-	-5565	526		2	2002	1227		
	AT	3553	366			Τ		2006	5031	5	ΑT	2002-	-7909	919		2	2002	1227		
	US	200	40248	3140		A1	L	2004	11209	9	US	2004-	-5003	346		2	20040	0729		
PRI	ORIT:	API	PLN.	INFO	).:						JP	2001-	-3969	941		A 2	2001	1227		
											WO	2002-	-JP13	3756		W 2	2002	1227		

ED Entered STN: 11 Jul 2003

AB A method of preparing a polynucleotide containing a nucleotide sequence which regulate the translation efficiency of a template in a protein synthesis system, comprising (a) applying a template containing one or more arbitrary nucleotide sequences to a protein synthesis reaction system, (b) after reacting, recovering a polynibosome fraction from the liquid reaction mixture, and (c) collecting a polynucleotide contg. the nucleotide sequence in the template from the polynibosome fraction; novel polynucleotides regulating the translation efficiency obtained by the above method; a method of synthesizing a protein with the use of a template containing such a polynucleotide; and so on. Use of d. gradient centrifugation for collecting polyribosome fraction is claimed. Isolation of 27 57nt randomized sequences and 96 22nt randomized sequences are reported.

REFERENCE COUNT: 8 THERE ARE 8 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 87 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2003:235544 CAPLUS  $\underline{\text{Full-text}}$ 

DOCUMENT NUMBER: 138:316222

TITLE: Cell-free protein synthesis by wheat germ extracts AUTHOR(S): Tozawa, Yuzuru; Sawasaki, Tatsuya; Endo, Yaeta

CORPORATE SOURCE: Mitsubishi Kagaku Inst. Life Sci., Yokohama, 227-8502,

Japan

SOURCE: Nippon Kessho Gakkaishi (2003), 45(1), 3-8

CODEN: NKEGAF; ISSN: 0369-4585

PUBLISHER: Nippon Kessho Gakkai DOCUMENT TYPE: Journal; General Review

LANGUAGE: Japanese ED Entered STN: 27 Mar 2003

AB A review. With the sequencing of the genomes of various species, attention has turned to the structure, properties, and functional activities of proteins. However, rapid progress in the area of proteomics is premised on the availability of sufficient amts. of a large number of proteins. Here we described a novel cell-free system from wheat embryos for the high-throughput screening/synthesis of gene products. Our system should open up many possibilities in the post-genome era.

ANSWER 88 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2003:123069 CAPLUS <u>Full-text</u>

138:148662 DOCUMENT NUMBER:

TITLE: Stabilization of nucleic acid template in cell-free

protein synthesis

Sawasaki, Tatsuya; Ogasawara, Tomio; Endo, Yaeta INVENTOR(S):

PATENT ASSIGNEE(S): Japan

Jpn. Kokai Tokkyo Koho, 18 pp. SOURCE:

CODEN: JKXXAF

DOCUMENT TYPE: Patent Japanese LANGUAGE:

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2003047496	A	20030218	JP 2001-238697	20010807
PRIORITY APPLN. INFO.:			JP 2001-238697	20010807

ED Entered STN: 18 Feb 2003

Substrates for nucleic acid-degrading enzyme(s) are used in the cell-free AB protein synthesis system to stabilize the nucleic acid template. The substrates are selected from nucleic acids that does not have the starting codon AUG, that do not code for the protein of interest, and that have low transcription/translation activity in the cell-free protein synthesis system. Enhanced expression of GFP gene in a wheat germ cell-free protein synthesis system in the presence of AUG-free GFP RNA was shown.

ANSWER 89 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN 2002:906658 CAPLUS Full-text ACCESSION NUMBER:

A1

DOCUMENT NUMBER: 137:366016

Method for screening plant embryo for cell-free protein TITLE: synthesis, and method for producing embryo extract for cell-free protein synthesis

INVENTOR(S): Endo, Yaeta; Iwahashi, Shigeo; Nomura, Kazuo

PATENT ASSIGNEE(S): Japan

SOURCE: PCT Int. Appl., 37 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 3

PATENT INFORMATION:

EP 1388733

	PA:	TENT NO.	KIND	DATE	APPLICATION NO.	DATE
	WO	2002095377	A1	20021128	WO 2002-JP4756	20020516
		W: AE, AG, AI	, AM, AT	, AU, AZ, BA	, BB, BG, BR, BY, BZ,	CA, CH, CN, CO,
CR,	CU,	CZ, DE, DK, DM,	DZ, EC,	EE, ES, FI,	GB, GD, GE, GH, GM,	HR, HU, ID, IL, IN,
IS,	ΚE,	KG, KP, KR, KZ,	LC, LK,	LR, LS, LT,	LU, LV, MA, MD, MG,	MK, MN, MW, MX, MZ,
		NO, NZ, ON	I, PH, PL	, PT, RO, RU	, SD, SE, SG, SI, SK,	SL, TJ, TM, TN,
TR,	TT,	TZ, UA, UG, US,	UZ, VN,	YU, ZA, ZM,	ZW	
		RW: GH, GM, KE	L, LS, MW	, MZ, SD, SL	, SZ, TZ, UG, ZM, ZW,	AT, BE, CH, CY,
DE,	DK,	ES, FI, FR, GB,	GR, IE,	IT, LU, MC,	NL, PT, SE, TR, BF,	BJ, CF, CG, CI, CM,
GΑ,	GN,	GQ, GW, ML, MR,	NE, SN,	TD, TG		
	JP	2002336797	A	20021126	JP 2001-149274	20010518
	JP	2002338597	A	20021127	JP 2001-149275	20010518
	AU	2002258191	A1	20021203	AU 2002-258191	20020516
	JP	2003315328	A	20031106	JP 2002-141141	20020516

20040211 EP 2002-728068

20020516

R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR

US 20040121462 A1 20040624 US 2004-478383 20040120 PRIORITY APPLN. INFO.: JP 2001-149274 A 20010518 JP 2001-149275 A 20010518 JP 2002-43704 A 20020220 WO 2002-JP4756 W 20020516

ED Entered STN: 29 Nov 2002

AB A method is provided for screening plant embryo for efficiently producing a germ extract for synthesizing a cell-free protein at a high synthesis efficiency. A method is also provided for producing a plant seed embryo extract for synthesizing a cell-free protein. Namely, the method for screening plant embryo for cell-free protein synthesis is characterized in that plant embryo is screened based on the optical information such as color information or image information, from a mixture containing embryo and ground albumen obtained by applying a mech. force to plant seeds (e.g., wheat, oat, rice). The method for producing a plant embryo extract is characterized in that the screened embryo is washed and finely ground, and afterwards, it is extracted

REFERENCE COUNT: 4 THERE ARE 4 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 90 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2002:900830 CAPLUS  $\underline{\text{Full-text}}$ 

DOCUMENT NUMBER: 137:348861

TITLE: Method for preparing an embryo bud extract for cell-free

protein synthesis, and cell-free protein synthesis method

INVENTOR(S): Endo, Yaeta; Ishibashi, Shigeo; Nomura, Kazuo

PATENT ASSIGNEE(S): Japan

SOURCE: Jpn. Kokai Tokkyo Koho, 9 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent LANGUAGE: Japanese

PATENT NO. KIND

Entered STN: 27 Nov 2002

ED

FAMILY ACC. NUM. COUNT: 3

PATENT INFORMATION:

					_					_				110.		_				
	JP	200	2338	597		А		2002	2112	7	JP	2001-	-1492	275		2	20010	518		
	WO	200	2095	377		A.	1	2002	21128	3	WO	2002-	JP47	756		2	20020	516		
		W:	ΑE	, AG,	, AL,	. AM	, AT,	AU	, AZ,	BA	, BB	, BG,	BR,	BY,	ΒZ,	CA,	CH,	CN,	CO,	
CR,	CU,	CZ,	DE,	DK,	DM,	DZ,	EC,	EE,	ES,	FI,	GB,	GD,	GE,	GH,	GM,	HR,	HU,	ID,	IL,	IN,
IS,	ΚE,	KG,	KP,	KR,	KΖ,	LC,	LK,	LR,	LS,	LT,	LU,	LV,	MA,	MD,	MG,	MK,	MN,	MW,	MX,	MZ,
			NO	, NZ,	, OM,	PH,	, PL,	PT	, RO,	RU	, SD	, SE,	SG,	SI,	SK,	SL,	ТJ,	TM,	TN,	
TR,	TT,	TZ,	UA,	UG,	US,	UZ,	VN,	YU,	ZA,	ZM,	ZW									
		RW	: GH	, GM,	, KE,	LS	, MW,	MZ	, SD,	SL	, SZ	, TZ,	UG,	ZM,	ZW,	ΑT,	BE,	CH,	CY,	
DE,	DK,	ES,	FI,	FR,	GB,	GR,	ΙE,	ΙT,	LU,	MC,	NL,	PT,	SE,	TR,	BF,	ВJ,	CF,	CG,	CI,	CM,
GA,	GN,	GQ,	GW,	$\mathrm{ML}$ ,	MR,	ΝE,	SN,	TD,	ΤG											
	ΑU	200	2258:	191		Α.	1	2002	21203	3	AU	2002-	-2581	91		2	20020	516		
	EP	138	8733			A.	1	200	40213	L	EΡ	2002-	-7280	068		2	20020	516		
		R:	AT	, BE,	, СН,	DE,	, DK,	ES,	, FR,	GB	, GR	, IT,	LI,	LU,	NL,	SE,	MC,	PT,	IE,	
SI,	LT,	LV,	FΙ,	RO,	MK,	CY,	AL,	TR												
	CN	151	1254			А		200	4070	7	CN	2002-	-8097	706		2	20020	516		
	US	200	4012	1462		A.	1	200	40624	1	US	2004-	-4783	883		2	20040	120		
PRI	CTIAC	Y AP	PLN.	INFO	0.:						JΡ	2001-	-1492	274		A 2	20010	518		
											JΡ	2001-	-1492	275		A 2	20010	518		
											JΡ	2002-	-4370	4		A 2	20020	220		
											WO	2002-	-JP47	756		W 2	20020	516		

DATE APPLICATION NO. DATE

An efficient method is provided for preparing an embryo bud extract for the AΒ cell-free protein synthesis with a high synthesis efficiency. A method is also provided for efficiently synthesizing a protein with the cell-free translation system on an industrial scale. The method for preparing an embryo bud extract comprises a process for grinding plant seeds (e.g., wheat, oat, rice, corn, spinach) and processes for selecting/washing/finely grinding/extracting the plant embryo bud. The selection of the plant embryo bud is characteristically performed using a color classifier.

ANSWER 91 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN 2002:416629 CAPLUS Full-text ACCESSION NUMBER:

137:43626 DOCUMENT NUMBER:

Post-genome era. Development of cell-free protein TITLE:

synthesis system and its applications

AUTHOR(S): Endo, Yaeta; Ogasawara, Tomio; Sawasaki, Tatsuva

CORPORATE SOURCE: Department of Applied Chemistry, Faculty of Engineering,

Ehime University, Matsuyama, 790-8577, Japan

Kinoshi Kenkyu Kaishi (2002), Volume Date 2001, 40, 69-73 SOURCE:

CODEN: KIKKDD; ISSN: 0288-5867

PUBLISHER: Kinoshi Kenkyukai

DOCUMENT TYPE: Journal; General Review

LANGUAGE: Japanese ED Entered STN: 04 Jun 2002

A review. Materialization of genetic information is an essential step toward AΒ modern biol. in both basic and applied fields today. We reported a novel system for massive production of gene products, which is based on wheat germ cell-free translation system. The methodol. consists of; (1) preparation of a highly efficient but also robust cell-free system, and (2) construction of a cell-free expression vector specialized for massive production of proteins. The method developed allowed to show that the system has high performance for materialization of genetic information directly from cDNA library. The possible applications of the system in the post-genome era are also discussed in this paper.

ANSWER 92 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN 2002:368678 CAPLUS Full-text ACCESSION NUMBER:

DOCUMENT NUMBER: 136:336855

TITLE: Process for producing germ extract Endo, Yaeta; Yamamoto, Masaharu INVENTOR(S):

PATENT ASSIGNEE(S): Wakenyaku Co., Ltd., Japan SOURCE: PCT Int. Appl., 31 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent Japanese LANGUAGE:

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO. KIND DATE APPLICATION NO. DATE \_\_\_\_ 20020516 WO 2001-JP9778 WO 2002038790 A1 W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CO, CR, CU, CZ, DE, DK, DM, DZ, EC, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PH, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, TR, BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG

JP 2002204689	А	20020723	JP	2000-341398		20001109
AU 2002012730	A5	20020521	AU	2002-12730		20011108
US 20040043088	A1	20040304	US	2003-416089		20030527
PRIORITY APPLN. INFO.:			JP	2000-340576	A	20001108
			JP	2000-341398	A	20001109
			WO	2001-JP9778	W	20011108

ED Entered STN: 18 May 2002

AB A efficient and low-cost process for producing a germ extract material characterized by involving the step of gently milling starting plant seeds to thereby eliminate the albumen of the seeds, the step of sieving the milled seeds under shaking to thereby recover fractions passing through 1.00-0.45 mm sieves, the step of eliminating the periderm of the seeds by winnowing, the step of recovering the suspension supernatant in water or an aqueous solution free from organic solvents (flotation), and the step of washing with water or an aqueous solution free from organic solvents; a germ extract material produced by this method; a germ extract obtained from this germ extract material; and a method of synthesizing a protein by using this germ extract The method does not use organic solvent, prevents unnecessary protein synthesis associated with phys. damages of the seed, and is useful for com. manufacture of enzymes and antibodies. Synthesis of green fluorescence protein (GFP) with wheat germ extract was shown.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 93 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2002:341321 CAPLUS <u>Full-text</u>

DOCUMENT NUMBER: 136:321725

TITLE: Use of apoprotein synthesized by cell-free system, and

method for producing holoprotein by cell-free system

INVENTOR(S): Kuroita, Toshihiro; Kawakami, Fumikiyo; Kawamura,

Yoshihisa; Nishikawa, Shigemichi; Endo, Yaeta

PATENT ASSIGNEE(S): Toyobo Co., Ltd., Japan; Wakenyaku Co., Ltd.

SOURCE: Jpn. Kokai Tokkyo Koho, 12 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2002125698	A	20020508	JP 2000-320108	20001019
PRIORITY APPLN. INFO.:			JP 2000-320108	20001019

ED Entered STN: 08 May 2002 AB A method and a reagent ar

AB A method and a reagent are provided for conveniently and accurately detecting or quantitating a different kind of cofactor or coenzyme. A convenient and efficient method is also provided for producing a holoprotein by a cell-free protein synthesis system. The reagent for detecting or quantitating a cofactor or a coenzyme contains an apoprotein (e.g., apoenzyme) synthesized by a cell-free protein synthesis system. The method for producing a holoprotein (e.g., holoenzyme) is characterized by synthesizing the protein in the presence of a cofactor or a coenzyme by a cell-free protein synthesis system. An example is shown with the holoenzyme of sarcosine oxidase synthesized by the wheat germ cell-free extract in the presence of FAD.

L7 ANSWER 94 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2002:341319 CAPLUS Full-text

DOCUMENT NUMBER: 136:336852

TITLE: Stabilization of freeze-dried cell extract for cell-free

protein synthesis

INVENTOR(S): Kuroita, Toshihiro; Kawakami, Fumikiyo; Kawamura,

Yoshihisa; Nishikawa, Shigemichi; Endo, Yaeta

PATENT ASSIGNEE(S): Toyobo Co., Ltd., Japan; Wakenyaku Co., Ltd.

SOURCE: Jpn. Kokai Tokkyo Koho, 14 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2002125693	A	20020508	JP 2000-320106	20001019
PRIORITY APPLN. INFO.:			JP 2000-320106	20001019

ED Entered STN: 08 May 2002

AB Inositol 0.3-3 weight% and polyalcs. 0.1-10, based on the weight of the protein in the cell ext., are useful for stabilization of freeze-dried cell extract for cell-free protein synthesis. The cell extract is obtained from wheat, barley, spinach, reticulocyte, Escherichia coli, etc. The cell extract may contain physiol. active substance selected from creatine kinase, pyruvate kinase, RMA polymerase, and chaperone proteins.

L7 ANSWER 95 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2002:240998 CAPLUS Full-text

DOCUMENT NUMBER: 136:243573

TITLE: Methods for cell-free protein synthesis

INVENTOR(S): Endo, Yaeta; Sawasaki, Tatsuya; Ogasawara, Tomio

PATENT ASSIGNEE(S): Wakenyaku Co., Ltd., Japan SOURCE: PCT Int. Appl., 33 pp.

В1

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

KR 749053

	PAT	rent	NO.			KIN	1D	DAT	E		APP	LICA	IION	NO.		Γ	DATE			
	WO	200	2024	939	_	 A1	 L	200	2032	3	WO	2001-	 -JP73	56		2	20010	828		
		W:	AE,	, AG	, AL,	, AM,	AT,	AU	, AZ	, BA,	, BB	, BG,	, BR,	BY,	BZ,	CA,	CH,	CN,	CO	,
CR,	CU,	CZ,	DE,	DK,	DM,	DZ,	EC,	EE,	ES,	FI,	GB,	GD,	GE,	GH,	GM,	HR,	HU,	ID,	IL,	IN,
IS,	JP,	ΚE,	KG,	KP,	KR,	KΖ,	LC,	LK,	LR,	LS,	LT,	LU,	LV,	MA,	MD,	MG,	MK,	MN,	MW,	MX,
			MZ	, NO	, NZ,	PH,	PL,	PT	, RO	, RU,	, SD	, SE,	, SG,	SI,	SK,	SL,	ТJ,	TM,	TR	,
TT,	TZ,	UA,	UG,	US,	UZ,	VN,	YU,	ZA,	ZW											
		RW	: GH	, GM	, KE,	LS,	MW,	MZ	, SD	, SL,	, SZ	, TZ,	, UG,	ZW,	ΑT,	BE,	CH,	CY,	DE	,
DK,	ES,	FI,	FR,	GB,	GR,	ΙE,	ΙΤ,	LU,	MC,	NL,	PT,	SE,	TR,	BF,	ВJ,	CF,	CG,	CI,	CM,	GA,
GN,	GQ,	GW,	ML,	MR,	NE,	SN,	TD,	ΤG												
	CA	242	0984			A1	L	200	2032	3	CA	2001-	-2420	984		2	20010	828		
	AU	200	1080	201		A		200	2040	2	AU	2001-	-8020	1		2	20010	828		
	EP	131	6617			A1	L	200	3060	4	EP	2001-	-9585	50		2	20010	828		
	EP	131	6617			В1	L	200	7080	1										
		R:	AT	, BE	, CH,	, DE,	DK,	ES	, FR	, GB,	, GR	, IT,	, LI,	LU,	NL,	SE,	MC,	PT,	ΙE	,
SI,	LT,	LV,	FI,	RO,	MK,	CY,	AL,	TR												
	CN	1449	9449			A		200	3101	5	CN	2001-	-8147	93		2	20010	828		
	JP	376	8190			В2	2	200	6041	9	JΡ	2002-	-5295	31		2	20010	828		
	AU	200	1280	201		В2	2	200	6071	3	AU	2001-	-2802	01		2	20010	828		
	AT	368	747			Τ		200	7081	5	ΑT	2001-	-9585	50		2	20010	828		
	CN	101	0924	46		А		200	7122	6	CN	2005-	-1009	0538	}	2	20010	828		

20070813 KR 2003-702765

20030225

US 20030162246	A1	20030828	US	2003-344803		20030318
US 6869774	В2	20050322				
JP 2006136330	A	20060601	JΡ	2005-355513		20051209
PRIORITY APPLN. INFO.:			JΡ	2000-259186	A	20000829
			CN	2001-814793	A3	20010828
			JΡ	2002-529531	A3	20010828
			WO	2001-JP7356	W	20010828

ED Entered STN: 28 Mar 2002

AΒ A batch-type method for synthesizing a cell-free protein which is characterized by comprising, in a means of a reaction for synthesizing a cellfree protein, (1) bringing a synthetic reaction solution (a reaction phase) containing a biol. extract into contact directly with a substrate and an energy source supplying solution (a supplying phase) without separating by a semi-permeable membrane, thus continuously supplying the substrate and the energy source mols. of the supplying phase into the reaction phase by free diffusion via the contact interface and, at the same time, removal of the byproducts formed in the reaction phase into the supplying phase to thereby prolong the reaction time, and thus elevating the reaction efficiency; (2) pre-incubating (pre-heating) a reaction solution, containing a wheat germ extract, then diluting the synthesis reaction solution by adding a diluent solution to thereby prolong the synthetic reaction time, and thus elevating the synthesis efficiency; (3) re-supplying a substrate required in the synthesis of the protein (amino acid, ATP, GTP, creatine phosphate, etc.) and an energy source to the reaction mixture with the use of a gel filtration column or a semi-permeable membrane after ceasing the synthesis reaction and, at the same time, discontinuously taking off the byproducts formed by the reaction to thereby elevate the efficiency of the reaction. Manufacture of green fluorescence protein (GFP) with wheat germ cell-free protein synthesis system containing plasmid pEU1 containing the gfp gene was shown. The production of the GFP was three time more than did the prior methods. method is useful for automation of and robot development for post genomic study of gene function.

REFERENCE COUNT: 7 THERE ARE 7 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 96 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2002:172088 CAPLUS Full-text

DOCUMENT NUMBER: 136:227877

TITLE: PCR primers for construction of transcription template for

dilution batch-type cell-free protein synthesis system

INVENTOR(S): Endo, Yaeta; Sawasaki, Tatsuya; Ogasawara, Tomio

PATENT ASSIGNEE(S): Wakenyaku Co., Ltd., Japan SOURCE: PCT Int. Appl., 66 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: Japanese

GN, GQ, GW, ML, MR, NE, SN, TD, TG

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

	PA:	TENT	NO.		_	KII	ND.	DATI	Ξ	_	APPI	LICAT	CION	NO.		I	DATE			
	WO	200	2018	586		Α.	1	2002	2030	7	WO 2	2001-	-JP73	357			2001	0828		
		W:	ΑE	, AG,	, AL,	AM,	, AT,	AU	, AZ	, BA,	, BB,	BG,	BR,	BY,	, BZ,	, CA	, CH	, CN,	, CO,	,
CR,	CU,	CZ,	DE,	DK,	DM,	DZ,	EC,	EE,	ES,	FI,	GB,	GD,	GE,	GH,	GM,	HR,	HU,	ID,	IL,	IN,
IS,	JP,	ΚE,	KG,	KP,	KR,	KΖ,	LC,	LK,	LR,	LS,	LT,	LU,	LV,	MA,	MD,	MG,	MK,	MN,	MW,	MX,
			MZ	, NO,	, NZ,	PH,	, PL,	PT,	, RO	, RU,	, SD,	SE,	SG,	SI,	, SK,	, SL	, TJ	, TM,	, TR	,
TT,	TZ,	UA,	UG,	US,	UΖ,	VN,	YU,	ZA,	ZW											
		RW	: GH	, GM,	, KE,	LS	, MW,	MZ	, SD	, SL,	, SZ,	TZ,	UG,	ZW,	, AT,	, BE	, CH	, CY,	, DE	,
DK,	ES,	FI,	FR,	GB,	GR,	ΙE,	ΙΤ,	LU,	MC,	NL,	PT,	SE,	TR,	BF,	ВJ,	CF,	CG,	CI,	CM,	GA,

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AU 2001080202
                      А
                             20020313 AU 2001-80202
                                                              20010828
    CA 2421012
                      A1
                             20030226 CA 2001-2421012
                                                              20010828
    EP 1314781
                       A1 20030528 EP 2001-958551
                                                              20010828
        R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE,
SI, LT, LV, FI, RO, MK, CY, AL, TR
                   В2
    AU 2001280202
                             20061109
                                        AU 2001-280202
                                                              20010828
                                        US 2003–363372 20030227
JP 2000–261638 A 20000830
    US 20040121346
                      A1
                             20040624
PRIORITY APPLN. INFO.:
                                        JP 2001-58404
                                                          A 20010302
                                        WO 2001-JP7357
                                                          W 20010828
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ED Entered STN: 08 Mar 2002

Primer sequences for construction of templates for a cell-free protein AΒ synthesis system using wheat germ extract are described. The 3'-terminal PCR primer contains sequences complementary to the sequence between the transcriptional termination sequence of a reporter gene (for example, a drugresistance gene) of the vector and Ori. The 5'-terminal primer contains sequences complementary to part of the promoter sequence. Those two types of primers satisfy the requirement of not priming the transcription from a DNA constructed by using only of those primers alone. One of those having a sequence complementary to part of the RNA polymerase recognition site from the 5'-terminus of the promoter and another having a sequence complementary to part of the RNA polymerase recognition site from the 3'-terminus of the promoter are provided as primers for 5'-terminal PCR. GA or GAA sequences is ligated to those sequences, and further downstream, transcription initiation codon ATG, part of the target gene ORF. A histidine tag, glutathione-Stransferase (GST), or myb tag or epitope preparation sequence may also be used. The reaction mixture is to diluted achieve the optimal magnesium concentration Cell-free protein synthesis using a modified mRNA having a tobacco mosaic virus (TMV)  $\boldsymbol{\Omega}$  sequence ligated to the 5'-end and an untranslated region (UTR) ligated to the 3'-end, is described.

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 97 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2002:90248 CAPLUS <u>Full-text</u>

DOCUMENT NUMBER: 136:114559

TITLE: Labeling of protein in a cell- and embryo-free wheat germ

protein synthesis system

INVENTOR(S): £ndo, Yaeta; Kumar, Penmetcha; Nishikawa, Shigemichi PATENT ASSIGNEE(S): National Institute of Advanced Industrial Science and

Technology, Japan; Wakenyaku Co., Ltd.

SOURCE: PCT Int. Appl., 42 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

	PA	TENT	NO.			KII	ND.	DAT	E		APP	LICAT	NOIT	NO.		I	DATE			
					_					_										
	WO	200	2008	443		A.	1	200	2013	1	WO 2	2001-	-JP62	226		2	2001	0718		
		W:	ΑE	, AG	, AL	, AM	, AT	, AU	, AZ	, BA	, BB	, BG,	BR,	BY,	BZ,	CA,	, CH	, CN,	, CO,	,
CR,	CU,	CZ,	DE,	DK,	DM,	DZ,	EC,	EE,	ES,	FI,	GB,	GD,	GE,	GH,	GM,	HR,	HU,	ID,	IL,	IN,
IS,	JP,	ΚE,	KG,	KP,	KR,	KΖ,	LC,	LK,	LR,	LS,	LT,	LU,	LV,	MA,	MD,	MG,	MK,	MN,	MW,	MX,
			MZ	, NO	, NZ	, PL	, PT	, RO	, RU	, SD	, SE	, SG,	SI,	SK,	SL,	, TJ,	, TM	, TR,	, TT,	,
TZ,	UA,	UG,	US,	UZ,	VN,	YU,	ZA,	ZW												
		RW	: GH	, GM	, KE	, LS	, MW	, MZ	, SD	, SL	, SZ	, TZ,	UG,	ZW,	AT,	, BE,	, CH	, CY,	, DE,	,
DK,	ES,	FI,	FR,	GB,	GR,	ΙE,	ΙΤ,	LU,	MC,	NL,	PT,	SE,	TR,	BF,	ΒJ,	CF,	CG,	CI,	CM,	GΑ,
GN,	GQ,	GW,	ML,	MR,	NE,	SN,	TD,	ΤG												

CA 2416684 A1 20020131 CA 2001-2416684 20010718

AU 2001072748 А 20020205 AU 2001-72748 20010718 EP 1310564 Α1 20030514 EP 2001-951912 20010718 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL, TR В2 AU 2001272748 20060831 AU 2001-272748 20010718 US 2003-333417 US 20030162245 A1 20030828 20030317 US 7074595 В2 20060711 

 JP 2000-220127
 A 20000721

 JP 2000-306119
 A 20001005

 WO 2001-JP6226
 W 20010718

 PRIORITY APPLN. INFO.: Entered STN: 01 Feb 2002 ED Selenomethionine-labeled protein is prepared with a wheat embryo cell-free AΒ wheat germ protein synthesis system by using selenomethionine-containing amino acids instead of methionine-containing amino acids, and protein synthesis under dialysis condition and batch conditions. Moreover, a process for producing a deuterium-labeled protein by using the same procedure was provided. Labeling of green fluorescent protein (GFP) and dihydrofolate reductase (DHFR) with the selenomethionine and deuterium was shown. The activity of the proteins was not altered by the labeling. REFERENCE COUNT: 13 THERE ARE 13 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT ANSWER 98 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2001:284088 CAPLUS Full-text DOCUMENT NUMBER: 134:306148 Cell-free protein synthesis using a modified mRNA having a TITLE: TMV  $\Omega$  sequence or alfalfa mosaic virus leader sequence INVENTOR(S): Endo, Yaeta PATENT ASSIGNEE(S): Wakenyaku Co., Ltd., Japan SOURCE: PCT Int. Appl., 82 pp. CODEN: PIXXD2 DOCUMENT TYPE: Patent LANGUAGE: Japanese FAMILY ACC. NUM. COUNT: 1 PATENT INFORMATION: KIND APPLICATION NO. DATE PATENT NO. DATE \_\_\_\_\_ \_\_\_\_ -----\_\_\_\_\_ A1 20010419 WO 2000-JP7123 WO 2001027260 20001013 W: AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW RW: GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG CA 2385134 A1 20010419 CA 2000-2385134 20001013 AU 2000-76861 AU 2000076861 Α 20010423 20001013 20020710 EP 2000-966474 EP 1221481 20001013 A1 R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO, MK, CY, AL JP 2005247857 20050915 JP 2005-78255 20050317 A PRIORITY APPLN. INFO.: JP 1999-294370 A 19991015 WO 2000-JP4814 A 20000718 JP 2001-530465 A3 20001013

ED Entered STN: 20 Apr 2001

AB Cell-free protein synthesis using a modified mRNA having a tobacco mosaic virus (TMV)  $\Omega$  sequence or alfalfa mosaic virus (AMV) leader sequence ligated

WO 2000-JP7123

W 20001013

to the 5'-end and an untranslated region (UTR) ligated to the 3'-end, is disclosed. A plasmid for the RNA synthesis comprising a promoter and a terminator is claimed. A means for continuous cell-free protein synthesis using endosperm-free wheat germ extract, where transcription and translation takes place sequentially, under dialysis, possibly using a porous filter, is claimed. Use of the cell-free protein synthesis method in evaluation of the relationship between genetic polymorphism and gene function, and screening of gene function, is claimed. Cell-free synthesis of various proteins, dihydrofolate reductase (dhfr), glutathione-S-transferase (GST), green fluorescent protein (GFP), GST-osteopontin fusion protein, luciferase, etc., is described.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 99 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2000:814649 CAPLUS <u>Full-text</u>

DOCUMENT NUMBER: 133:331186

TITLE: Cell-free protein synthesis and preparation for cell-free

protein synthesis

INVENTOR(S): Endo, Yaeta; Nishikawa, Shigemichi

PATENT ASSIGNEE(S): Wakenyaku Co., Ltd., Japan SOURCE: PCT Int. Appl., 50 pp.

CODEN: PIXXD2

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DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

	PA:	CENT						DATE		API	PLICAT	CION	NO.		D	ATE			
	WO	2000						2000	1116	WO	1999-	 -JP40	88		1	9990	 729		
		W:	ΑU,	CA,	CN,	IL,	KR	, US,	AM,	AZ, B	Y, KG,	KΖ,	MD,	RU,	ТJ,	TM			
		RW:	AT,	BE,	CH,	CY,	DE,	, DK,	ES,	FI, F	R, GB,	GR,	ΙE,	ΙT,	LU,	MC,	NL,	PT,	SE
	JΡ	2000	3165	94		Α		2000	1121	JP	1999-	-1303	93		1	9990	511		
	JΡ	2000	3165	95		Α		2000	1121	JP	1999-	-1303	95		1	9990	511		
	JΡ	2000	3336	73		Α		2000	1205	JP	1999-	-1515	99		1	9990	531		
	CA	2373	3057			A1		2000	1116	CA	1999-	-2373	057		1	9990	729		
	ΑU	9949	301			Α		2000	1121	AU	1999-	-4930	1		1	9990	729		
	ΑU	7656	32			В2		2003	0925										
	EP	1176	210			A1		2002	0130	EP	1999-	-9331	68		1	9990	729		
		R:	AT,	BE,	CH,	DE,	DK,	, ES,	FR,	GB, G	R, IT,	LI,	LU,	NL,	SE,	MC,	PT,	ΙE,	FI
	US	6905	843			В1		2005	0614	US	2001-	-1999	5		2	0011	120		
	US	2005	0186	655		A1		2005	0825	US	2005-	-8965	2		2	0050	325		
	US	7235	382			В2		2007	0626										
	JΡ	2005	3487	39		Α		2005	1222	JP	2005-	-2030	81		2	0050	712		
PRIOR	RITY	APP	LN.	INFO	.:					JP	1999-	-1303	93		A 1	9990	511		
										JP	1999-	-1303	95		A 1	9990	511		
										JP	1999-	-1515	99		A 1	9990	531		
										WO	1999-	-JP40	88		W 1	9990	729		
										US	2001-	-1999	5		A3 2	0011	120		
	_																		

3 DD1 103 E1011 110

ED Entered STN: 21 Nov 2000

AB A preparation for synthesizing a cell-free protein which contains a cell extract prepared by eliminating inhibition system or inhibitors of self-protein synthesis; an apparatus for synthesizing a cell-free protein provided with a reaction tank for synthesizing the cell-free protein; and a kit to be used therefor are given. The above prepn. is obtained as a product which can be stored at room temperature while sustaining the biol. functions of the cell extract A means for continuously synthesizing a cell-free protein comprising a cell extract from which substances inhibiting self-protein synthesis reactions have been substantially eliminated, involving a procedure selected

from among addition, preservation, exchange, and discharge of a factor selected from among mRNA template in the synthesis reactions, an enzyme associated with the energy regeneration system, a substrate, and an energy source. Preparation of luciferase and other protein with wheat germ extract pretreated with nonionic surfactant NP-40 and then subjected to tritin removal was shown.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L7 ANSWER 100 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2000:616369 CAPLUS <u>Full-text</u>

DOCUMENT NUMBER: 133:173704

TITLE: Cell-free protein synthesis using wheat germ extract

INVENTOR(S): Endo, Yaeta

PATENT ASSIGNEE(S): Mitsubishi Chemical Corp., Japan; Cellfree Sciences Co.,

Ltd.

SOURCE: Jpn. Kokai Tokkyo Koho, 10 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

	PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
	JP 2000236896	——— А	20000905	JP 1999-46379	19990224
	JP 3753358	В2	20060308	01 1333 10073	19990111
	JP 2005312460	A	20051110	JP 2005-196673	20050705
PRIOF	RITY APPLN. INFO.:			JP 1999-46379	A3 19990224

ED Entered STN: 06 Sep 2000

AB Embryo-free plant germ exts. are used for cell-free protein synthesis. The embryo-free plant germ exts. are free of endogenous inhibitors such as tritin and thionin. The plant germ is selected from wheat, barley, rice, and corn. The germ extract is prepared by sonication in the presence of surfactant and/or formycin-5'-phosphate. Amino acid, and energy source are supplied for the manufacture of proteins.

L7 ANSWER 101 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2000:421640 CAPLUS Full-text

DOCUMENT NUMBER: 133:330997

TITLE: The construction of highly efficient cell-free protein

synthesis from wheat embryos

AUTHOR(S): Endo, Yaeta

CORPORATE SOURCE: Japan

SOURCE: Bio Industry (2000), 17(5), 20-27 CODEN: BIINEG; ISSN: 0910-6545

PUBLISHER: Shi Emu Shi

DOCUMENT TYPE: Journal; General Review

LANGUAGE: Japanese ED Entered STN: 23 Jun 2000

AB A review with 14 refs. on the cell-free protein synthesis with wheat germ. It comprises optimization of cell-free protein synthesis, instability and low translation initiation in the cell-free system, and development of higherficiency cell-free system from wheat germ.,.

L7 ANSWER 102 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 1992:610746 CAPLUS Full-text

DOCUMENT NUMBER: 117:210746

ORIGINAL REFERENCE NO.: 117:36393a,36396a

TITLE: Cell-free, continuous synthesis of polypeptide INVENTOR(S): Yokoyama, Shigeyuki; Endo, Yaeta; Kikawa, Takanori

PATENT ASSIGNEE(S): Japan

SOURCE: Jpn. Kokai Tokkyo Koho, 7 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 04200390	A	19920721	JP 1990-334103	19901130
PRIORITY APPLN. INFO.:			JP 1990-334103	19901130

ED Entered STN: 28 Nov 1992

AB A cell-free, ribosome-containing, continuous system for synthesis of polypeptide (excluding chem. synthesis) from mRNA is disclosed. In this system, the reaction substrates, e.g., ATP, GTP, amino acids, etc., are continuously supplied to the reaction chamber having minimal air content and the reaction mixture is pumped through an ultrafiltration apparatus to sep. polypeptide products from the low-mol. substrates. The process minimizes foaming and therefore protein denaturation as compared to that of prior art. Synthesis of chloramphenicol acetyltransferase (CAT) was performed continuously for 17 h with a good yield.

L7 ANSWER 103 OF 103 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 1979:166178 CAPLUS <u>Full-text</u>

DOCUMENT NUMBER: 90:166178

ORIGINAL REFERENCE NO.: 90:26371a,26374a

TITLE: lpha-Fetoprotein synthesis in yolk sac tumor: sex-dependent

production of  $\alpha\text{-fetoprotein}$  by transplantable rat yolk sac tumor

AUTHOR(S): Endo, Y.; Kaneko, Y.; Urano, Y.; Tsuchida, Y.; Watabe, H.;

Tsukada, Y.; Sakashita, S.; Hirai, H.; Oda, T.

CORPORATE SOURCE: Dep. Med., Univ. Tokyo, Tokyo, Japan

SOURCE: Scandinavian Journal of Immunology, Supplement (1978),

8(Carcinoembryonic Proteins: Recent Prog.), 181-6

CODEN: SJISDK; ISSN: 0301-6323

DOCUMENT TYPE: Journal LANGUAGE: English ED Entered STN: 12 May 1984

AB Expts. at cellular and subcellular levels were caried out. In cell incubation studies, yolk sac tumor cells maintained in female rat (YST-F cells) synthesized more α-fetoprotein (AFP) than yolk sac tumor cells maintained in male rat (YST-M cells). AFP production was studied in cell-free proteinsynthesizing systems derived from wheat germ, using tumor RNA. In this system, cytosol RNA from both YST-F and YST-M cells directed AFP synthesis. But the amount of AFP synthesized was smaller in the presence of RNA from YST-M cells. The reduced AFP synthesis by YST-M cells may be due to a quant. decrease in their cytosol mRNA coded for AFP.

<sup>=&</sup>gt; s 16 AND ( freeze-dried OR freeze-drying OR freeze(2a)(dried OR drying) OR lyophiliz?)

L8 6 L6 AND (FREEZE-DRIED OR FREEZE-DRYING OR FREEZE(2A)(DRIED OR DRYING) OR LYOPHILIZ?)

## => d ibib ed abs 18 1-6

L8 ANSWER 1 OF 6 BIOSIS COPYRIGHT (c) 2008 The Thomson Corporation on STN

ACCESSION NUMBER: 2006:589439 BIOSIS Full-text

DOCUMENT NUMBER: PREV200600593047

TITLE: Composition for cell-free protein synthesis.

AUTHOR(S): Anonymous; Kuroita, Toshihiro [Inventor]; Kawakami, Bunsei [Inventor]; Kawamura, Yoshihisa [Inventor]; Nishikawa, Shiqemichi [Inventor]; Endo,

Yaeta [Inventor]

CORPORATE SOURCE: Tsuruga, Japan

ASSIGNEE: CellFree Sciences Co Ltd

PATENT INFORMATION: US 07048915 20060523

SOURCE: Official Gazette of the United States Patent and Trademark

Office Patents, (MAY 23 2006)

CODEN: OGUPE7. ISSN: 0098-1133.

DOCUMENT TYPE: Patent LANGUAGE: English

ENTRY DATE: Entered STN: 8 Nov 2006

Last Updated on STN: 8 Nov 2006

ED Entered STN: 8 Nov 2006

Last Updated on STN: 8 Nov 2006

AB The present invention provides a composition for cell-free protein synthesis, which is superior in storage stability in a freeze-dried state, more particularly a freeze-dryable or freeze-dried composition for cell-free protein synthesis, which contains a cell extract for cell-free protein synthesis and inositol, and a freeze-dryable or freeze-dried composition for cell-free protein synthesis containing a cell extract for cell-free protein synthesis, and a deliquescent material in a proportion of not more than 0.01 part by weight per part by weight of a protein in the composition; and a composition for cell-free protein synthesis superior in storage stability in a frozen state, more particularly a freezable or frozen composition for cell-free protein synthesis, containing a cell extract for cell-free protein synthesis and polyhydric alcohol.

L8 ANSWER 2 OF 6 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2007:428031 CAPLUS Full-text

DOCUMENT NUMBER: 146:375315

TITLE: Freeze-dried template for cell-free protein synthesis and

laboratory application

INVENTOR(S): Endo, Yaeta; Sawasaki, Tatsuya; Tanaka, Michihiro;

Morishita, Akira; Saeki, Mihoro

PATENT ASSIGNEE(S): Cellfree Sciences Co., Ltd., Japan SOURCE: Jpn. Kokai Tokkyo Koho, 27pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2007097438	A	20070419	JP 2005-288613	20050930
PRIORITY APPLN. INFO.:			JP 2005-288613	20050930

ED Entered STN: 19 Apr 2007

AB The freeze-dried template is nucleotide sequence for visible proteins selected from green fluorescent protein, blue fluorescent protein, etc. The RNA polymerase and phosphotransferase such as creatine kinase used in the cell-free protein synthesis do not contain animal and microbial contaminants. With

proper solns., the freeze-dried template-containing cell-free protein synthesis system may be easily used, and protein synthesis monitored by the presence of visible proteins. Also, the cell-free protein synthesis system such as wheat germ extract does not use expansive RNase inhibitors.

ANSWER 3 OF 6 CAPLUS COPYRIGHT 2008 ACS on STN

ACCESSION NUMBER: 2003:913316 CAPLUS Full-text

DOCUMENT NUMBER: 139:360695

Lyophilized preparation for cell-free protein synthesis TITLE:

Endo, Yaeta; Ogasawara, Tomio INVENTOR(S):

PATENT ASSIGNEE(S): Japan

SOURCE: PCT Int. Appl., 32 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

	PA'	TENT	NO.			KII	ND		<b>Ξ</b>		APP	LICAT	NOI	NO.		Ι	DATE			
	WO	200	3095	 661	_	A	1				WO	2003-	-JP56	556		2	20030	506		
		W:	AE,	, AG,	, AL,	, AM	, AT,	AU,	, AZ,	, BA	, BB	, BG,	BR,	BY,	BZ,	CA,	CH,	CN,	CO	,
CR,	CU,	CZ,	DE,	DK,	DM,	DZ,	EC,	EE,	ES,	FI,	GB,	GD,	GE,	GH,	GM,	HR,	HU,	ID,	IL,	IN,
IS,	JP,	ΚE,	KG,	KP,	KR,	KΖ,	LC,	LK,	LR,	LS,	LT,	LU,	LV,	MA,	MD,	MG,	MK,	MN,	MW,	MX,
			MZ	, NI,	, NO,	NZ,	, OM,	PH,	, PL,	PT.	, RO	, RU,	SC,	SD,	SE,	SG,	SK,	SL,	TJ	,
TM,	TN,	TR,	TT,	TZ,	UA,	UG,	US,	UZ,	VC,	VN,	YU,	ZA,	ZM,	ZW						
		RW	: GH	, GM,	, KE,	LS	, MW,	MZ,	, SD,	, SL	, SZ	, TZ,	UG,	ZM,	ZW,	AM,	AZ,	BY,	KG	,
KΖ,	MD,	RU,	ΤJ,	TM,	ΑT,	BE,	BG,	CH,	CY,	CZ,	DE,	DK,	EE,	ES,	FΙ,	FR,	GB,	GR,	HU,	ΙE,
ΙΤ,	LU,	MC,	NL,	PT,	RO,	SE,	SI,	SK,	TR,	BF,	ВJ,	CF,	CG,	CI,	CM,	GΑ,	GN,	GQ,	GW,	ML,
			MR	, NE	, SN,	TD	, TG													
	AU	200.	3235	844		A.	1	2003	31111	1	AU	2003-	-2358	344		2	20030	)506		
	CA	248	5827			A.	1	2003	31120	)	CA	2003-	-2485	827		2	20030	506		
	EP	155	0728			A.	1	2005	50706	5	EΡ	2003-	-7210	30		2	20030	506		
		R:	AT	, BE	, CH,	DE	, DK,	ES,	FR,	, GB	, GR	, IT,	LI,	LU,	NL,	SE,	MC,	PT,	ΙE	,
SI,	LT,											HU,		·	·	·	·	·		
·			•						•			2004-		355		2	20041	1221		
PRI				INF								2002-					20020	)514		
												2003-								

Entered STN: 21 Nov 2003 ED

The lyophilized cell-free protein synthesis system has comparable activity to AΒ that prepared by low-temperature preservation. The cell-free protein synthesis system contains lower deliquescent substances such as potassium acetate. The deliquescent substances amount to  $\leq 0.01$  weight% based on 1 weight% protein in the cell-free protein synthesis system. The low-mol. weight protein synthesis inhibitors are removed in the presence of high-energy phosphate compds. such as ATP.

REFERENCE COUNT: 9 THERE ARE 9 CITED REFERENCES AVAILABLE FOR THIS RECORD, ALL CITATIONS AVAILABLE IN THE RE FORMAT

ANSWER 4 OF 6 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2003:836518 CAPLUS <u>Full-text</u> DOCUMENT NUMBER: 139:319681

Composition for cell-free protein synthesis TITLE:

Kuroita, Toshihiro; Kawakami, Bunsei; Kawamura, Yoshihisa; INVENTOR(S):

Nishikawa, Shigemichi; Endo, Yaeta

PATENT ASSIGNEE(S): Toyo Boseki Kabushiki Kaisha, Japan; Wakenyaku Co., Ltd.;

Cellfree Sciences Co., Ltd.

SOURCE: U.S. Pat. Appl. Publ., 20 pp.

CODEN: USXXCO

DOCUMENT TYPE: Patent LANGUAGE: English

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
US 20030199076	A1	20031023	US 2002-124953	20020418
US 7048915	B2	20060523		
PRIORITY APPLN. INFO.:			US 2002-124953	20020418

ED Entered STN: 24 Oct 2003

AB The present invention provides a composition for cell-free protein synthesis, which is superior in storage stability in a freeze-dried state, more particularly a freeze-dryable or freeze-dried composition for cell-free protein synthesis, which contains a cell extract for cell-free protein synthesis and inositol, and a freeze-dryable or freeze-dried composition for cell-free protein synthesis containing a cell extract for cell-free protein synthesis, and a deliquescent material in a proportion of not more than 0.01 part by weight per part by weight of a protein in the composition; and a composition for cell-free protein synthesis superior in storage stability in a frozen state, more particularly a freezable or frozen composition for cell-free protein synthesis, containing a cell extract for cell-free protein synthesis and polyhydric alc.

REFERENCE COUNT: 6 THERE ARE 6 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

L8 ANSWER 5 OF 6 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2002:341319 CAPLUS Full-text

DOCUMENT NUMBER: 136:336852

TITLE: Stabilization of freeze-dried cell extract for cell-free

protein synthesis

INVENTOR(S): Kuroita, Toshihiro; Kawakami, Fumikiyo; Kawamura,

Yoshihisa; Nishikawa, Shigemichi; Endo, Yaeta

PATENT ASSIGNEE(S): Toyobo Co., Ltd., Japan; Wakenyaku Co., Ltd.

SOURCE: Jpn. Kokai Tokkyo Koho, 14 pp.

CODEN: JKXXAF

DOCUMENT TYPE: Patent LANGUAGE: Japanese

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION:

PATENT NO.	KIND	DATE	APPLICATION NO.	DATE
JP 2002125693	А	20020508	JP 2000-320106	20001019
PRIORITY APPLN. INFO.:			JP 2000-320106	20001019

ED Entered STN: 08 May 2002

AB Inositol 0.3-3 weight% and polyalcs. 0.1-10, based on the weight of the protein in the cell ext., are useful for stabilization of freeze-dried cell extract for cell-free protein synthesis. The cell extract is obtained from wheat, barley, spinach, reticulocyte, Escherichia coli, etc. The cell extract may contain physiol. active substance selected from creatine kinase, pyruvate kinase, RMA polymerase, and chaperone proteins.

L8 ANSWER 6 OF 6 CAPLUS COPYRIGHT 2008 ACS on STN ACCESSION NUMBER: 2000:814649 CAPLUS Full-text

DOCUMENT NUMBER: 133:331186

TITLE: Cell-free protein synthesis and preparation for cell-free

protein synthesis

INVENTOR(S): Endo, Yaeta; Nishikawa, Shigemichi

PATENT ASSIGNEE(S): Wakenyaku Co., Ltd., Japan

SOURCE: PCT Int. Appl., 50 pp.

CODEN: PIXXD2

DOCUMENT TYPE: Patent Japanese LANGUAGE:

FAMILY ACC. NUM. COUNT: 1

PATENT INFORMATION: 

	PA:	PATENT NO.						DATE		APPLICATION NO.					DATE						
	WO	2000068412					20001116		,	wo	1999-JP4088			8		19990729					
		W:	ΑU,	CA,	CN,	IL,	KR,	US,	AM,	ΑZ,	ВҮ	, KG,	K.	Z,	MD,	RU,	ТJ,	TM			
		RW:	ΑT,	BE,	CH,	CY,	DE,	DK,	ES,	FΙ,	FR	, GB,	GI	R,	ΙE,	ΙΤ,	LU,	MC,	NL,	PT,	SE
	JΡ	P 2000316594				A		2000	1121	JP 1999-130393							19990511				
	JP 2000316595				Α		2000	1121	JP 1999-130395							19990511					
	JΡ	2000	3336	73		Α		2000	1205	1	JΡ	1999-	-15	159	9		-	9990	531		
	CA	2373	057			A1		2000	1116	1	CA	1999-	-23	730	57		_	9990	729		
	AU	9949	301			Α		2000	1121		AU	1999-	-493	301			_	9990	729		
	AU	7656	32			В2		2003	0925												
	EP	1176	210			A1		2002	0130		ΕP	1999-	-933	316	8		_	9990	729		
		R:	ΑT,	BE,	CH,	DE,	DK,	ES,	FR,	GB,	GR	, IT,	L.	I,	LU,	NL,	SE,	MC,	PT,	ΙE,	FI
	US	6905	843			В1		2005	0614		US	2001-	-199	995	ı		2	20011	120		
	US	2005	0186	655		A1		2005	0825		US	2005-	-896	652			2	20050	325		
	US	7235	382			В2		2007	0626												
	JΡ	2005	3487	39		A		2005	1222	1	JΡ	2005-	-203	308	1		2	20050	712		
PRIO	PRIORITY APPLN. INFO.:										JΡ	1999-	-130	039	3		A 1	9990	511		
										1	JΡ	1999-	-130	039	5		A 1	9990	511		
										1	JΡ	1999-	-15:	159	9		A 1	9990	531		
										,	WO	1999-	-JP	408	8		W 1	9990	729		
												2001-									

ED Entered STN: 21 Nov 2000

AΒ A preparation for synthesizing a cell-free protein which contains a cell extract prepared by eliminating inhibition system or inhibitors of selfprotein synthesis; an apparatus for synthesizing a cell-free protein provided with a reaction tank for synthesizing the cell-free protein; and a kit to be used therefor are given. The above prepn. is obtained as a product which can be stored at room temperature while sustaining the biol. functions of the cell extract A means for continuously synthesizing a cell-free protein comprising a cell extract from which substances inhibiting self-protein synthesis reactions have been substantially eliminated, involving a procedure selected from among addition, preservation, exchange, and discharge of a factor selected from among mRNA template in the synthesis reactions, an enzyme assocd. with the energy regeneration system, a substrate, and an energy source. Preparation of luciferase and other protein with wheat germ extract pretreated with nonionic surfactant NP-40 and then subjected to tritin removal was shown.

REFERENCE COUNT: 10 THERE ARE 10 CITED REFERENCES AVAILABLE FOR THIS RECORD. ALL CITATIONS AVAILABLE IN THE RE FORMAT

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(FILE 'HOME' ENTERED AT 14:59:34 ON 04 JUN 2008)

FILE 'MEDLINE, BIOSIS, CAPLUS, EMBASE' ENTERED AT 14:59:49 ON 04 JUN 2008 E ENDO Y/AU

L12996 S E3-E7

E SAWASAKI T/AU

91 S E3 L2

202 S E11-E12 L3

L4 3093 S L1-L3
L5 215 S L4 AND ((PROTEIN(2A)SYNTHES?) OR TRANSLATION)(3A)((IN(A)VITRO) OR CELL(A)FREE OR CELL-FREE)
L6 121 DUP REM L5 (94 DUPLICATES REMOVED)
L7 103 S L6 AND (MRNA OR TEMPLATE OR WHEAT OR ATP)
L8 6 S L6 AND (FREEZE-DRIED OR FREEZE-DRYING OR FREEZE(2A)(DRIED OR DRYING) OR LYOPHILIZ?)

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